FIGURE 1A

1 CGGATGCTGC TGCTACTGTC ACTTCTGCCG CTGCCGCTGT TGTTACAGAT

51 TTTGCTTTTG CTCCTTCTAC CGCATGACAA TTGTTTTCCT CGCCTAAGCA

101 GATACCAGCC TCAGATGCTC AAGGTGAGAG TCTTGCCTTT CGCTCTGGGC

151 TATTGGTTCA CTTAATCCGG TCAATTTGTT CGCTGCTCGT GGTTGTCTTT

201 CTCCCCGCCC TCCTTCCCCC TGTTTTGTTT TGTTTCGCTT GCTTTCGGGG

251 GGACGCTCCT TCCCTCAGTC AGAAGAGCTG GAATTGCTTG AGAGGCGTAT

301 AAGGAATTAT AAAAGTGGCC AGGAAACACG AGCGCAGTGA CTGCAGAGCT

351 GCCCTTGGCT TCGGCAAGGC AGCGTGAGCG GCAGAGGGCT CGGGCAGGGG

401 GCGGGGGGTC TCCTTTTTCC CGTGCGTTCC TCTTCTCCCA GTTCGGATGA

451 TGTTGCTGTT TCGGACCTCT CGCTGACTCC TGCCCTGTGA TTTTTGCTGA

501 GCGCTGTGAC TGTTACTCCG TCTCTTTCTG TCTGTGTTTC ACAGTAATGG

551 ACTGTGATAG AGTTAAGGCC TTTTGGAGGT GAGCTGTGTC ACAGCTGATG

601 CTTAAACATG TCTGAAGTAG GCACCGAGAC TTTCCCCAGC CCCTCGGCTC

651 AGCTGAGCCC TGATGCATCC CTTGGCGGGC TCCCGGCTGA GGAGAACATG

701 CCGGGGCCCC ACAGAGAGGA CAGCAGGGTC CCAGGTGTGG CAGGCCTGGC

751 CTCGACCTGC TGCGTGTGCC TGGAAGCAGA GCGACTGAAG GGCTGCCTCA

801 ACTCTGAGAA GATCTGCATC GCCCCTATCC TGGCTTGCCT GCTCAGCCTC

851 TGCCTCTGCA TTGCTGGCCT CAAGTGGGTC TTTGTGGACA AGATTTTTGA

901 GTATGACTCT CCTACACACC TTGACCCTGG GAGGATAGGA CAAGACCCAA

951 GGAGCACTGT GGATCCTACA GCTCTGTCTG CCTGGGTGCC TTCGGAGGTG

1001 TATGCCTCAC CCTTCCCCAT ACCTAGCCTT GAGAGCAAGG CTGAAGTGAC

1051 AGTGCAAACT GACAGCTCGC TCGTGCCCTC CAGGCCCTTC CTTCAGCCTT

1101 CTCTCTACAA CCGCATCCTA GATGTCGGGT TGTGGTCCTC TGCCACACCG

1151 TCACTGTCAC CATCCTCCCT GGAGCCTACC ACGGCATCTC AGGCACAAGC

1201 AACAGAAACC AATCTCCAAA CTGCTCCAAA ACTTTCCACT TCTACATCTA

1251 CAACTGGGAC AAGTCATCTC ACAAAATGTG ACATAAAGCA GAAAGCCTTC

1301 TGTGTAAATG GGGGAGAGTG CTACATGGTT AAAGACCTCC CAAACCCTCC

FIGURE 1B

```
1351  ACGATACCTA TGCAGGTGCC CAAATGAATT TACTGGTGAT CGCTGCCAAA
1401  ACTACGTAAT GGCCAGCTTC TACAAGCATC TTGGGATTGA ATTTATGGAA
1451  GCTGAGGAAC TGTACCAGAA ACGGGTGCTG ACCATAACTG GCATTTGCAT
1501  TGCTCTTCTA GTAGTTGGCA TCATGTGTGT GGTGGCCTAC TGCAAAACCA
1551  AGAAGCAGAG GAAAAAGTTG CATGACCGCC TTCGGCAGAG CCTTCGCTCA
1601  GAGAGGAACA ACGTTATGAA CATGGCAAAT GGGCCACACC ACCCCAACCC
1651  ACCACCAGAC AATGTCCAGC TGGTGAATCA GTACGTTTCA AAAAACATAA
1701  TCTCCAGTGA ACGTGTCGTT GAGCGAGAAA CCGAGACCTC GTTTTCCACA
1751  AGCCACTACA CCTCAACAAC TCATCACTCC ATGACAGTCA CCCAGACGCC
1801  TAGCCACAGC TGGAGTAATG GCCATACCGA AAGCATTCTC TCCGAAAGCC
1851  ACTCCGTGCT CGTCAGCTCC TCAGTGGAGA ATAGCAGGCA CACCAGCCCA
1901  ACAGGGCCAC GAGGCCGCCT CAATGGCATT GGTGGGCCAA GGGAAGGCAA
1951  CAGCTTCCTC CGGCATGCAA GAGAGACCCC TGACTCCTAC CGAGACTCTC
2001  CTCACAGTGA AAGGTATGTC TCAGCTATGA CCACACCAGC TCGCATGTCA
2051  CCCGTTGATT TCCACACTCC AACTTCTCCC AAGTCCCCTC CATCTGAAAT
2101  GTCACCACCA GTTTCCAGCT TGACCATCTC CATCCCTTCG GTGGCGGTGA
2151  GTCCCTTTAT GGACGAGGAG AGACCGCTGC TGTTGGTGAC CCCACCACGG
2201  CTGCGTGAGA AGTACGACAA CCACCTTCAG CAATTCAACT CCTTCCACAA
2251  CAATCCCACC CATGAGAGCA ACAGTCTGCC ACCCAGTCCT CTGAGGATAG
2301  TGGAGGATGA AGAGTATGAG ACCACGCAGG AGTACGAACC AGCACAGGAG
2351  CCTCCAAAGA AACTCACCAA CAGCCGGAGG GTGAAAAGAA CAAAGCCCAA
2401  TGGCCATATT TCCAGCAGGG TAGAAGTGGA CTCCGACACA AGCTCTCAGA
2451  GCACTAGCTC TGAGAGCGAA ACAGAAGATG AAAGAATAGG TGAGGATACA
2501  CCATTTCTTA GCATACAAAA TCCCATGGCA ACCAGTCTGG AGCCAGCCGC
2551  TGCATATCGG CTGGCTGAGA ACAGGACTAA CCCGGCAAAT CGCTTCTCCA
2601  CACCAGAAGA GTTGCAAGCA AGGTTGTCCA GTGTAATAGC TAACCAAGAC
2651  CCTATTGCTG TATAAGACAT AAACAAAACA CATAGATTCA CATGTAAAAC
```

FIGURE 1C

```
2701  TTTATTTTAT ATAATGAAGT ATTCCACCTT TAAATTAAAC AATTTATTTT
2751  ATTTTAGCAA TTCCGCTGAT AGAAAACAAG AGTGGAAAAA GAAACTTTTA
2801  TAAATTAAGT ATACGTATGT ACAAATGTGT TATGTGCCAT ATGTAGCAAT
2851  TTTTTACAGT ATTTCCAAAA TGGGGAAAGA TATCAATGGT GCCTTTATGT
2901  TATGTTATGT TGAGAGCAAG TTTTGTACAG CTACAATGAT TGCTGTCCCG
2951  TAGTATTTTG CAAAACCTTC TAGCCCTCAG TTGTTCTGGC TTTTTTGTGC
3001  ATTGCATTAT AATGACTGGA TGTATGATTT GCAAGAATTG CAGAAGTCCC
3051  CATTTGCTTG TTGTGGAATC CCCAGATCAA AAAGCCCTGT TATGGCACTC
3101  ACACCCTATC CACTTCACCA GGAAAAAAAA AAAATCAAAA AAAAAAAAAA
3151  AAAAAAAAGA AAAGAAAGAG AAAAAAGAAA AGAAAAAGAA AAAAAAAGCT
3201  GAAAAAATAA AA
```

FIGURE 2

```
   1  GCCCYCHFCR CRCCYRFCFC SFYRMTIVFL A*ADTSLRCS R*ESCLSLWA
  51  IGSLNPVNLF AARGCLSPRP PSPCFVLFRL LSGGRSFPQS EELELLERRI
 101  RNYKSGQETR AQ*LQSCPWL RQGSVSGRGL GQGAGGLLFP VRSSSPSSDD
 151  VAVSDLSLTP AL*FLLSAVT VTPSLSVCVS Q*WTVIELRP FGGELCHS*C
 201  LNMSEVGTET FPSPSAQLSP DASLGGLPAE ENMPGPHRED SRVPGVAGLA
 251  STCCVCLEAE RLKGCLNSEK ICIAPILACL LSLCLCIAGL KWVFVDKIFE
 301  YDSPTHLDPG RIGQDPRSTV DPTALSAWVP SEVYASPFPI PSLESKAEVT
 351  VQTDSSLVPS RPFLQPSLYN RILDVGLWSS ATPSLSPSSL EPTTASQAQA
 401  TETNLQTAPK LSTSTSTTGT SHLTKCDIKQ KAFCVNGGEC YMVKDLPNPP
 451  RYLCRCPNEF TGDRCQNYVM ASFYKHLGIE FMEAEELYQK RVLTITGICI
 501  ALLVVGIMCV VAYCKTKKQR KKLHDRLRQS LRSERNNVMN MANGPHHPNP
 551  PPDNVQLVNQ YVSKNIISSE RVVERETETS FSTSHYTSTT HHSMTVTQTP
 601  SHSWSNGHTE SILSESHSVL VSSSVENSRH TSPTGPRGRL NGIGGPREGN
 651  SFLRHARETP DSYRDSPHSE RYVSAMTTPA RMSPVDFHTP TSPKSPPSEM
 701  SPPVSSLTIS IPSVAVSPFM DEERPLLLVT PPRLREKYDN HLQQFNSFHN
 751  NPTHESNSLP PSPLRIVEDE EYETTQEYEP AQEPPKKLTN SRRVKRTKPN
 801  GHISSRVEVD SDTSSQSTSS ESETEDERIG EDTPFLSIQN PMATSLEPAA
 851  AYRLAENRTN PANRFSTPEE LQARLSSVIA NQDPIAV*DI NKTHRFTCKT
 901  LFYIMKYSTF KLNNLFYFSN SADRKQEWKK KLL*IKYTYV QMCYVPYVAI
 951  FYSISKMGKD INGAFMLCYV ESKFCTATMI AVP*YFAKPS SPQLFWLFCA
1001  LHYNDWMYDL QELQKSPFAC CGIPRSKSPV MALTPYPLHQ EKKKIKKKKK
1051  KKRKEREKRK EKEKKS*KNK
```

FIGURE 3

1 CGGCCTGTAA GATGCTGTAT CATTTGGTTG GGGGGGCCTC TGCGTGGTAA
51 TGGACCGTGA GAGCGGCCAG GCCTTCTTCT GGAGGTGAGC CGATGGAGAT
101 TTATTCCCCA GACATGTCTG AGGTCGCCGC CGAGAGGTCC TCCAGCCCCT
151 CCACTCAGCT GAGTGCAGAC CCATCTCTTG ATGGGCTTCC GGCAGCAGAA
201 GACATGCCAG AGCCCCAGAC TGAAGATGGG AGAACCCCTG GACTCGTGGG
251 CCTGGCCGTG CCCTGCTGTG CGTGCCTAGA AGCTGAGCGC CTGAGAGGTT
301 GCCTCAACTC AGAGAAAATC TGCATTGTCC CCATCCTGGC TTGCCTGGTC
351 AGCCTCTGCC TCTGCATCGC CGGCCTCAAG TGGGTATTTG TGGACAAGAT
401 CTTTGAATAT GACTCTCCTA CTCACCTTGA CCCTGGGGGG TTAGGCCAGG
451 ACCCTATTAT TTCTCTGGAC GCAACTGCTG CCTCAGCTGT GTGGGTGTCG
501 TCTGAGGCAT ACACTTCACC TGTCTCTAGG GCTCAATCTG AAAGTGAGGT
551 TCAAGTTACA GTGCAAGGTG ACAAGGCTGT TGTCTCCTTT GAACCATCAG
601 CGGCACCGAC ACCGAAGAAT CGTATTTTTG CCTTTTCTTT CTTGCCGTCC
651 ACTGCGCCAT CCTTCCCTTC ACCCACCCGG AACCCTGAGG TGAGAACGCC
701 CAAGTCAGCA ACTCAGCCAC AAACAACAGA AACTAATCTC CAAACTGCTC
751 CTAAACTTTC TACATCTACA TCCACCACTG GGACAAGCCA TCTTGTAAAA
801 TGTGCGGAGA AGGAGAAAAC TTTCTGTGTG AATGGAGGGG AGTGCTTCAT
851 GGTGAAAGAC CTTTCAAACC CCTCGAGATA CTTGTGCAAA GGCGGAGGAG
901 CTGTACCAGA AGAGAGTGCT GACCATAACC GGCATCTGCA TCGCCCTCCT
951 TGTGGTCGGC ATCATGTGTG TGGTGGCCTA CTGCAAAACC AAGAAACAGC
1001 GGAAAAAGCT GCATGACCGT CTTCGGCAGA GCCTTCGGTC TGAACGAAAC
1051 AATACGATGA ACATTGCCAA TGGGCCTCAC CATCCTAACC CACCCCCCGA
1101 GAATGTCCAG CTGGTGAATC AATACGTATC TAAAAACGTC ATCTCCAGTG
1151 AGCATATTGT TGAGAGAGAA GCAGAGACAT CCTTTTCCAC CAGTCACTAT
1201 ACTTCCACAG CCCATCACTC CACTACTGTC ACCCAGACTC CTAGCCACAG
1251 CTGGAGCAAC GGACACACTG AAAGCATCCT TTCCGAAAGC CACTCTGTAA
1301 TCGTGATGTC ATCCGTAGAA AACAGTAGGC ACAGCAGCCC AACTGGGGCC
1351 G

FIGURE 4

1 ACKMLYHLVG GASAW*WTVR AARPSSGGEP MEIYSPDMSE VAAERSSSPS

51 TQLSADPSLD GLPAAEDMPE PQTEDGRTPG LVGLAVPCCA CLEAERLRGC

101 LNSEKICIVP ILACLVSLCL CIAGLKWVFV DKIFEYDSPT HLDPGGLGQD

151 PIISLDATAA SAVWVSSEAY TSPVSRAQSE SEVQVTVQGD KAVVSFEPSA

201 APTPKNRIFA FSFLPSTAPS FPSPTRNPEV RTPKSATQPQ TTETNLQTAP

251 KLSTSTSTTG TSHLVKCAEK EKTFCVNGGE CFMVKDLSNP SRYLCKGGGA

301 VPEESADHNR HLHRPPCGRH HVCGGLLQNQ ETAEKAA*PS SAEPSV*TKQ

351 YDEHCQWASP S*PTPRECPA GESIRI*KRH LQ*AYC*ERS RDILFHQSLY

401 FHSPSLHYCH PDS*PQLEQR TH*KHPFRKP LCNRDVIRRK Q*AQQPNWG

FIGURE 5A

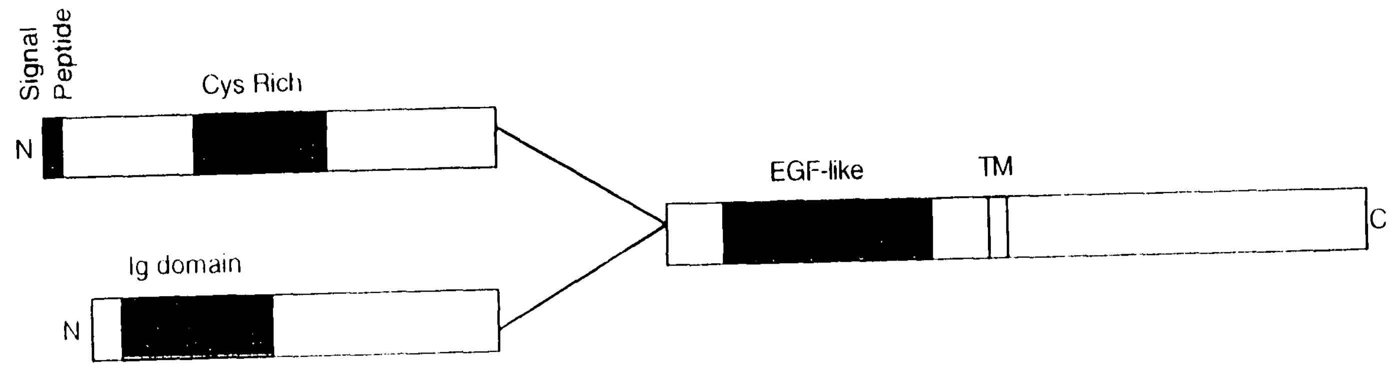


FIGURE 5B

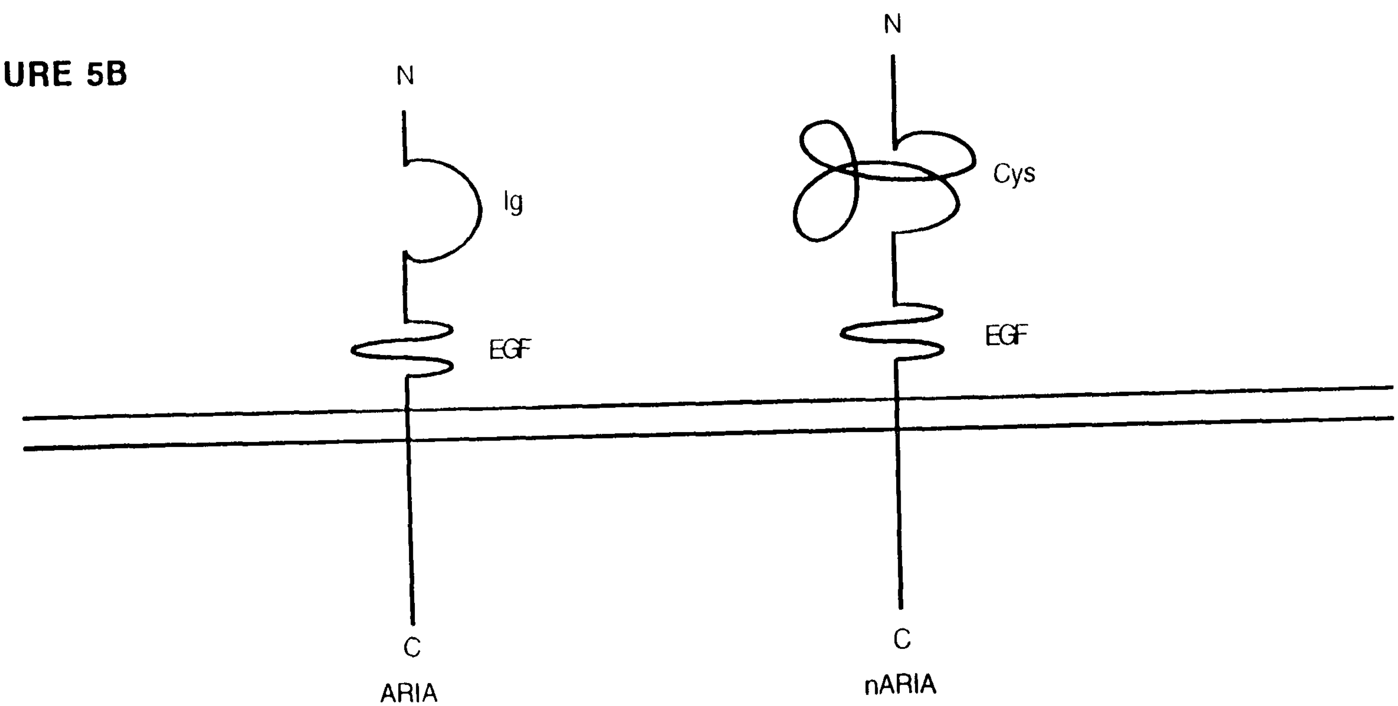


FIGURE 6

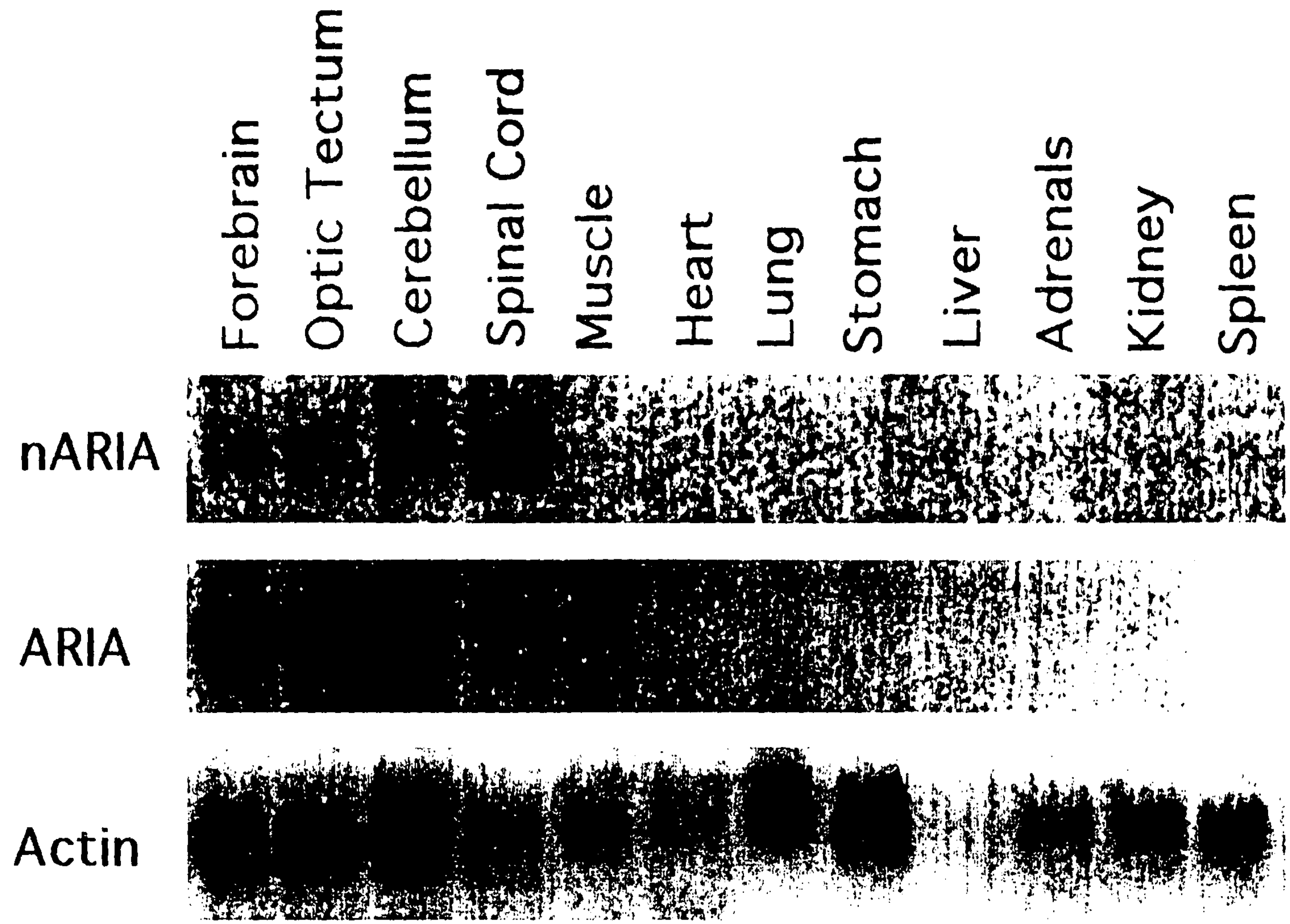

Forebrain
Optic Tectum
Cerebellum
Spinal Cord
Muscle
Heart
Lung
Stomach
Liver
Adrenals
Kidney
Spleen
nARIA
ARIA
Actin

FIGURE 7

Developmental Northern of ARIA and nARIA in the chick hindbrain and cerebellum

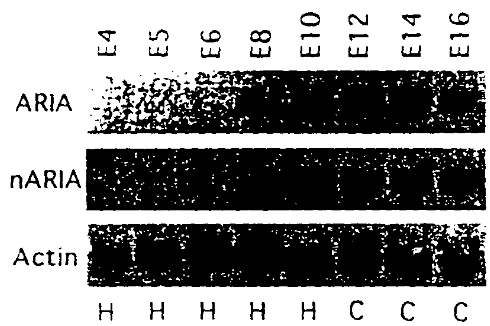


FIGURE 8A

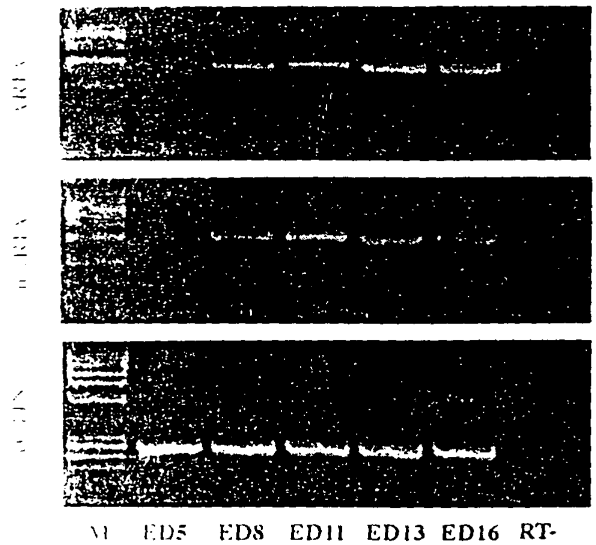


FIGURE 8B

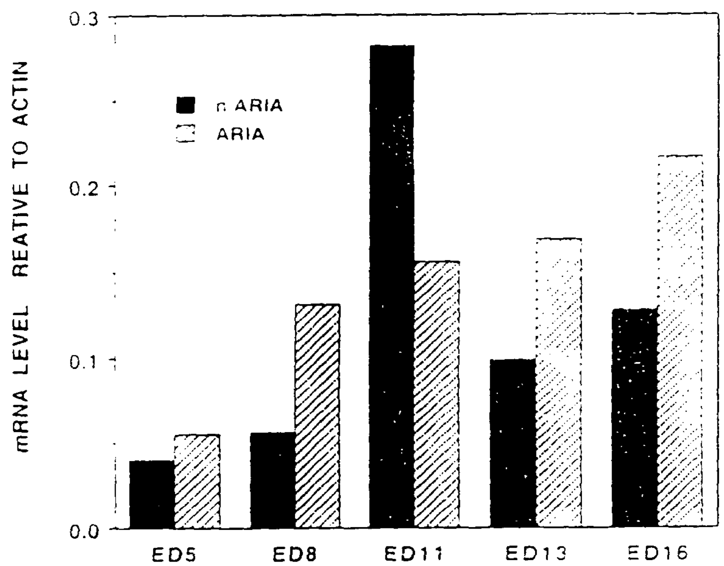

0.3
0.2
0.1
0.0
mRNA LEVEL REATIVE TO ACTIN
n ARIA
ARIA
ED5
ED8
ED11
ED13
ED16

FIGURE 9A

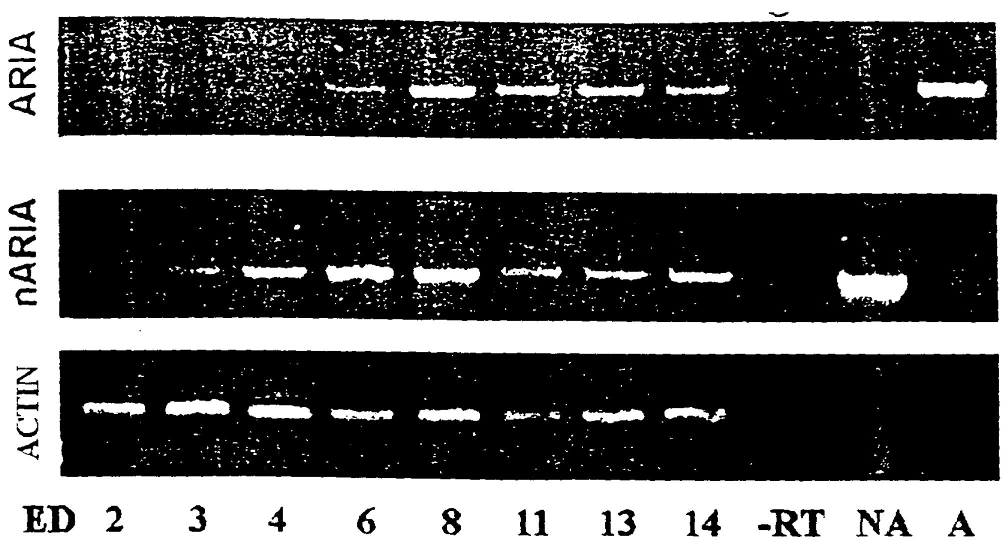


FIGURE 9B

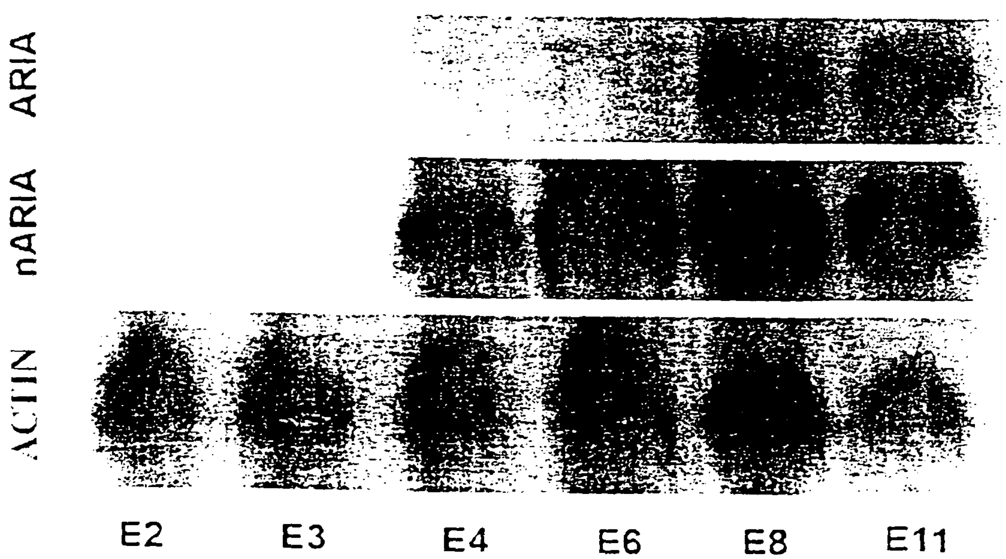


FIGURE 9C

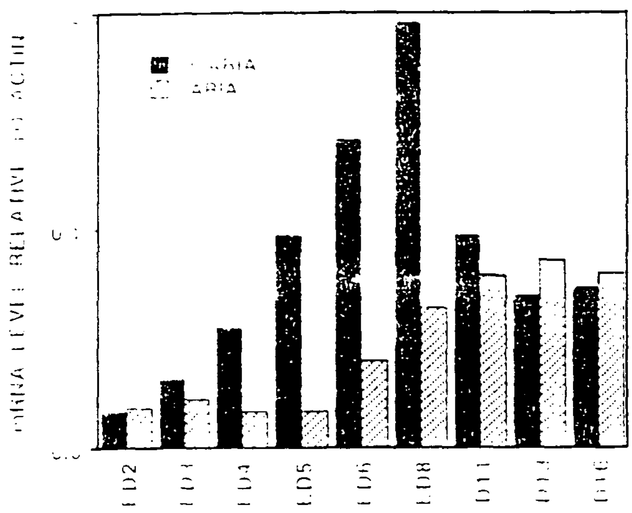
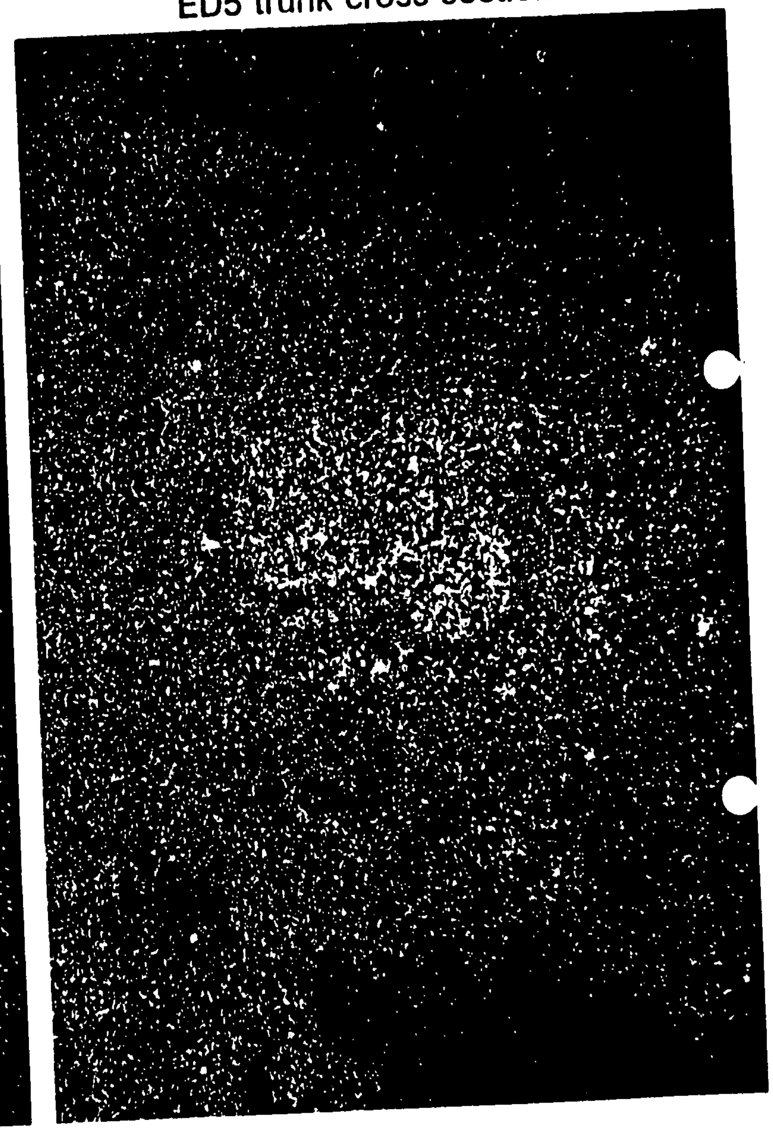


FIGURE 10A
ED5 trunk cross-section

nARIA specific probe

FIGURE 10B
ED5 trunk cross-section



ARIA specific probe

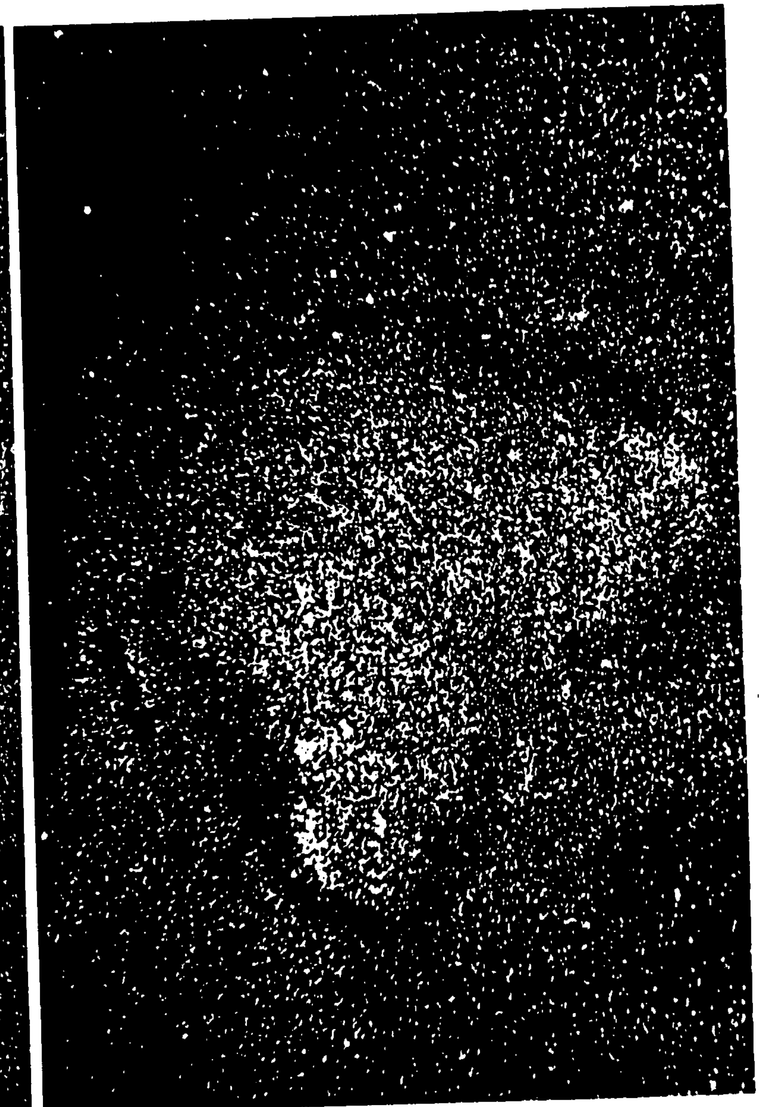
FIGURE 10C

nARIA specific probe

ED7 trunk cross-section

FIGURE 10D

ARIA specific probe



ED7 trunk cross-section

FIGURE 11A

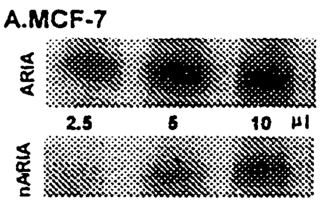


FIGURE 11B

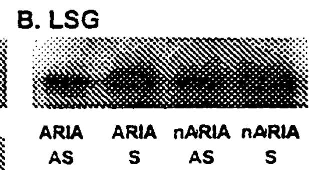


FIGURE 11C

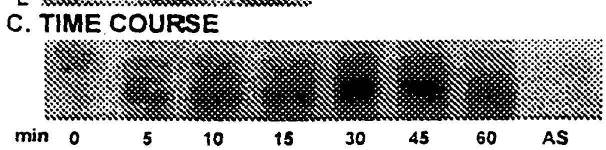


FIGURE 12

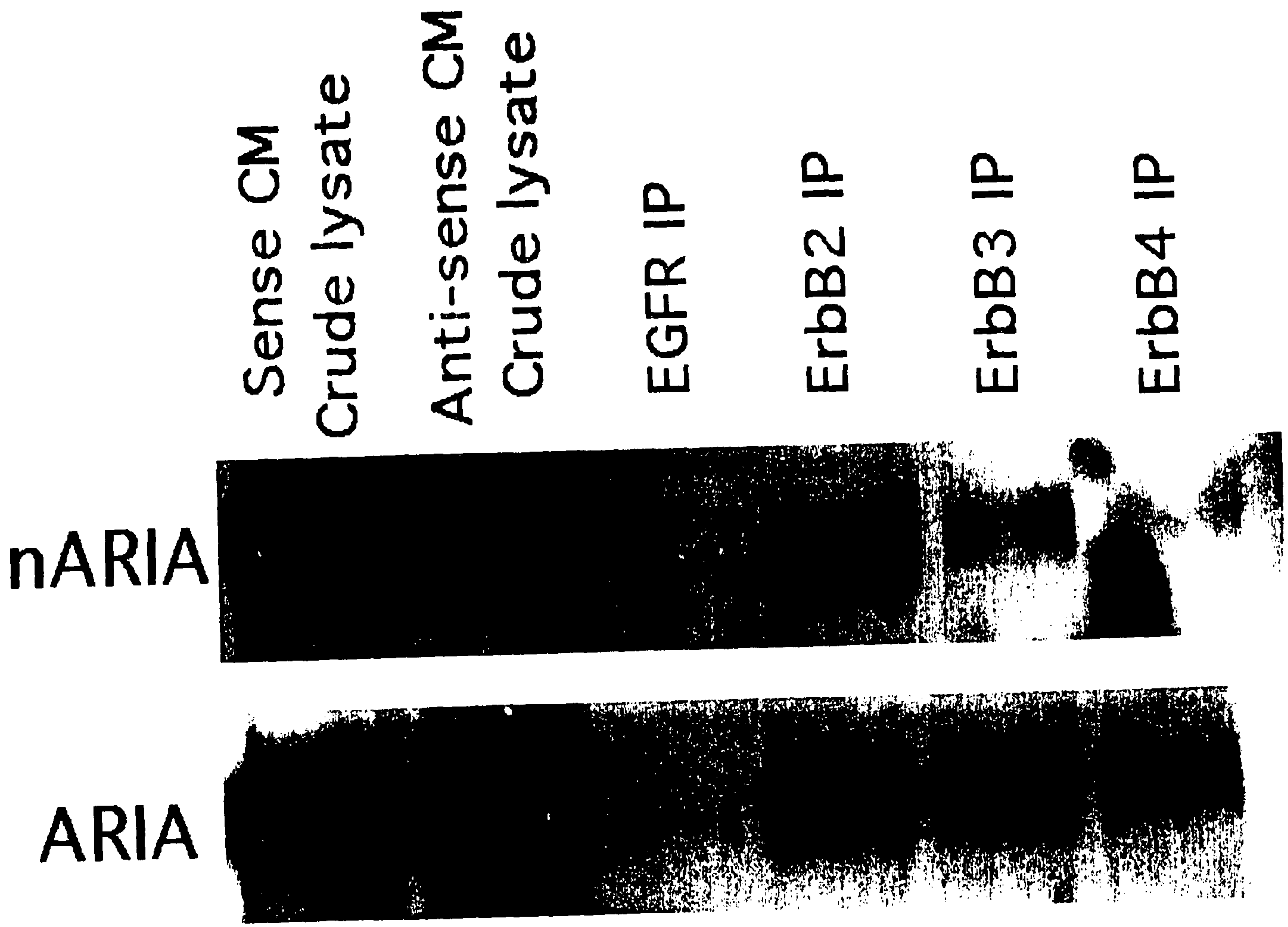

Sense CM
Crude lysate
Anti-sense CM
Crude lysate
EGFR IP
ErbB2 IP
ErbB3 IP
ErbB4 IP
nARIA
ARIA

FIGURE 13A

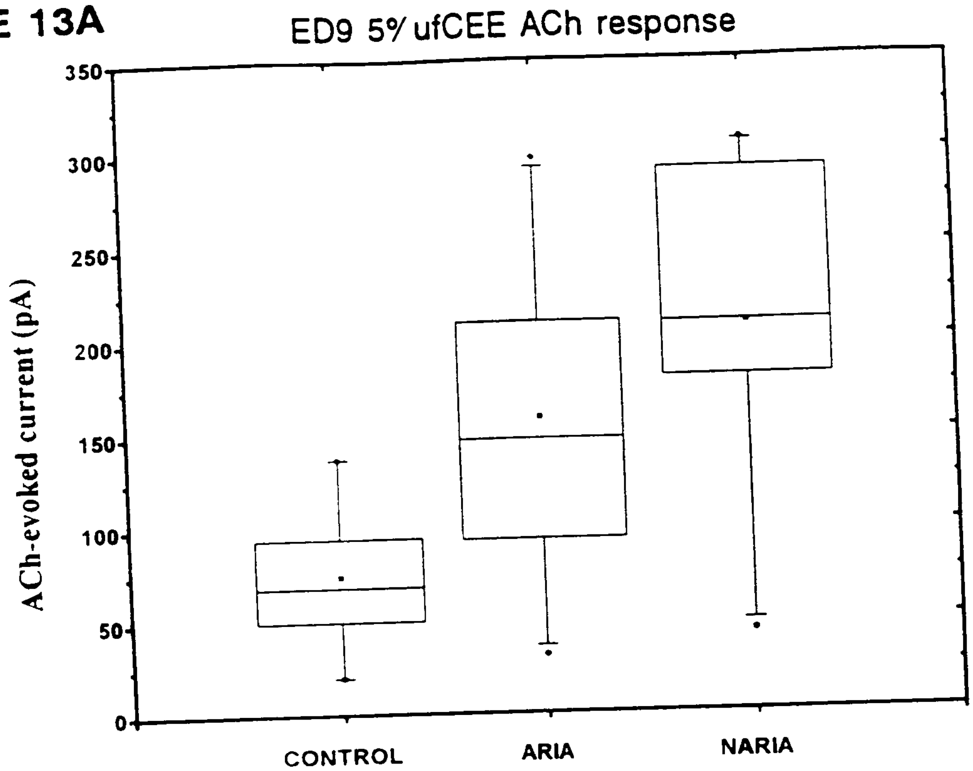


FIGURE 13B

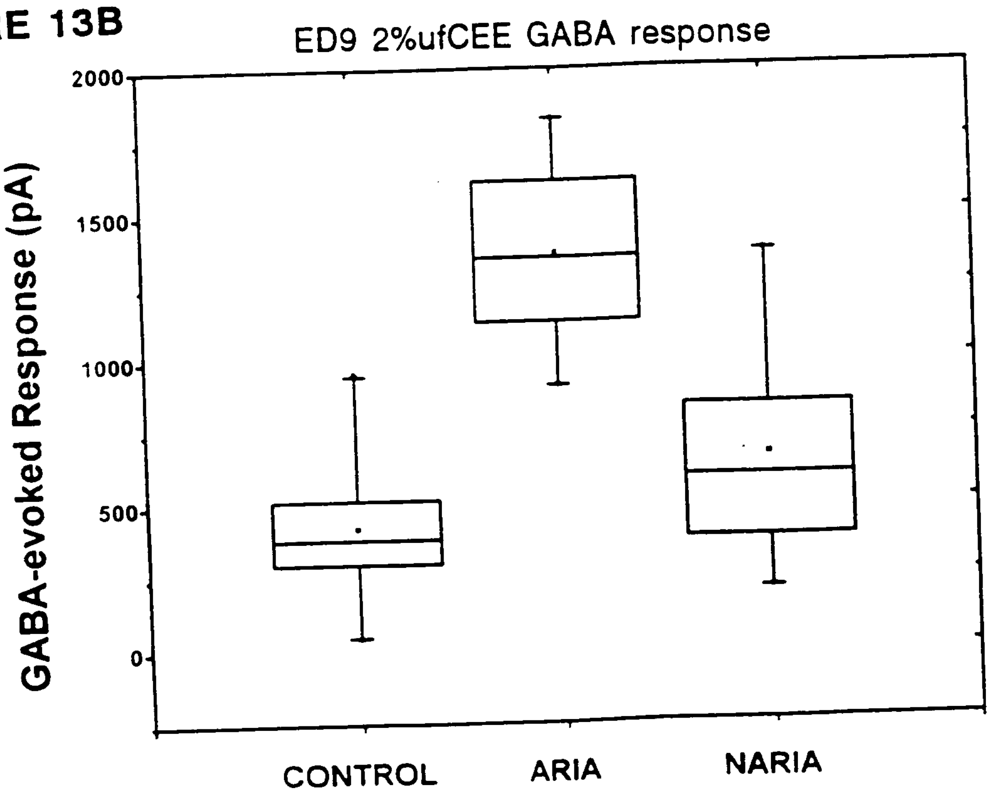


FIGURE 13C

ED 11

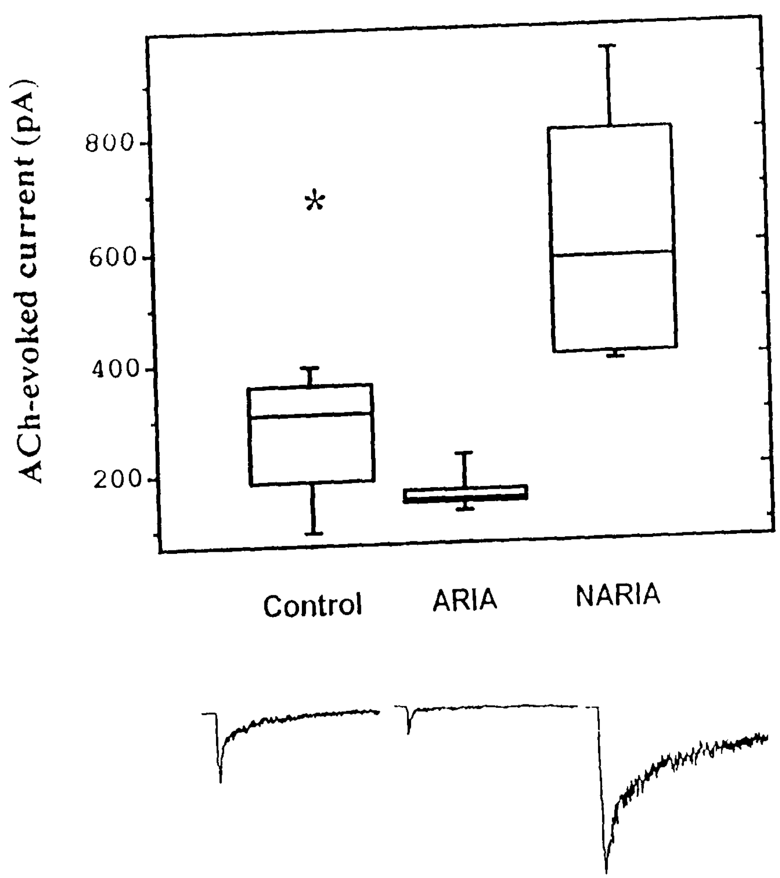


FIGURE 13D

ED11

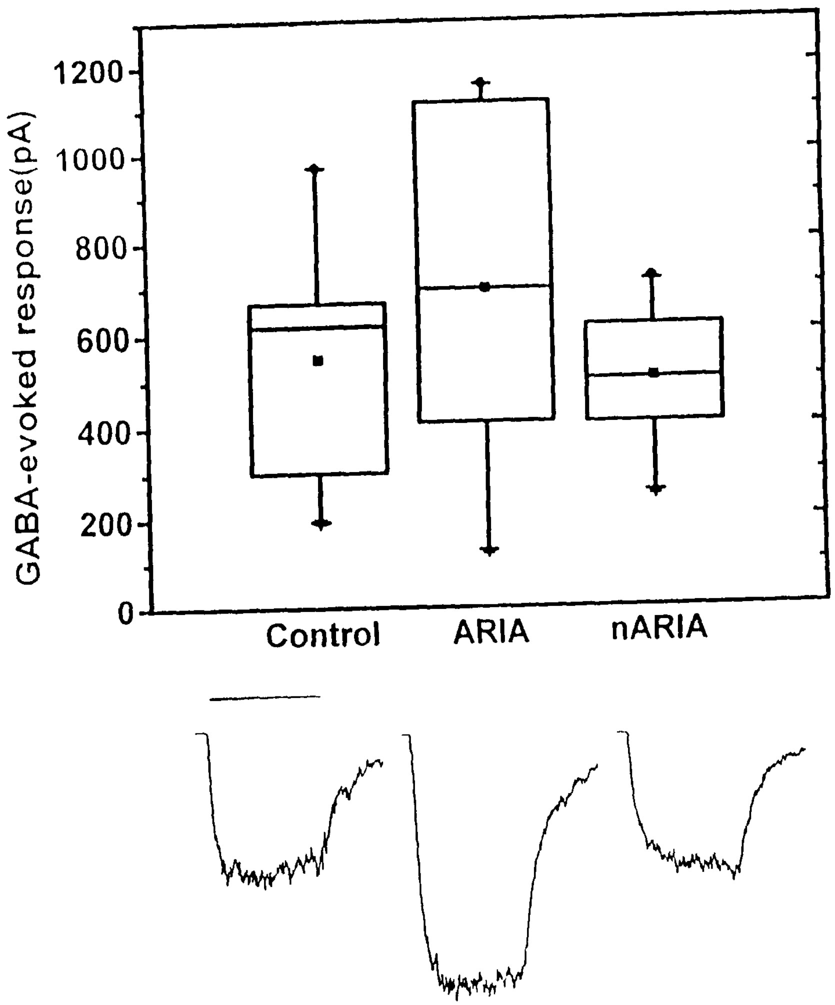


FIGURE 14A

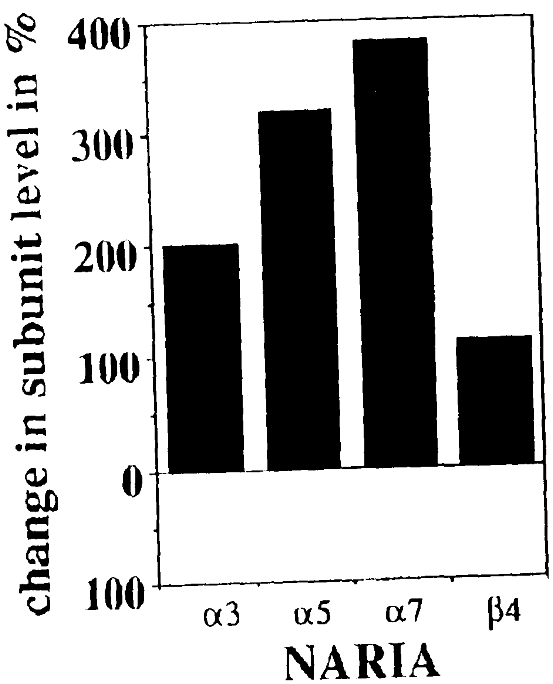


FIGURE 14B

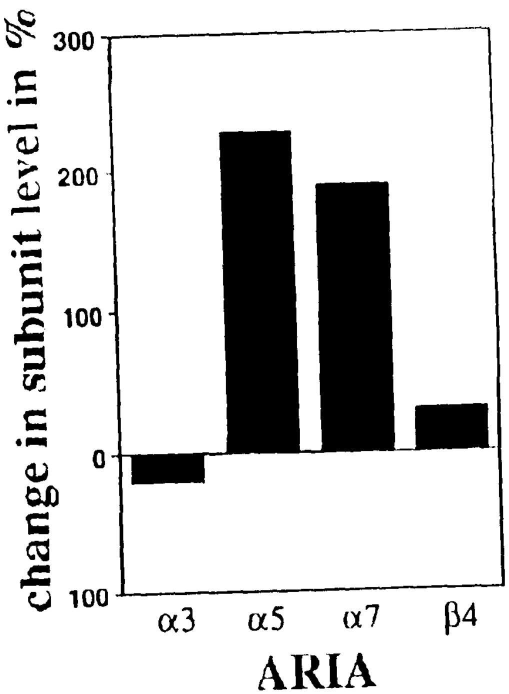


FIGURE 15

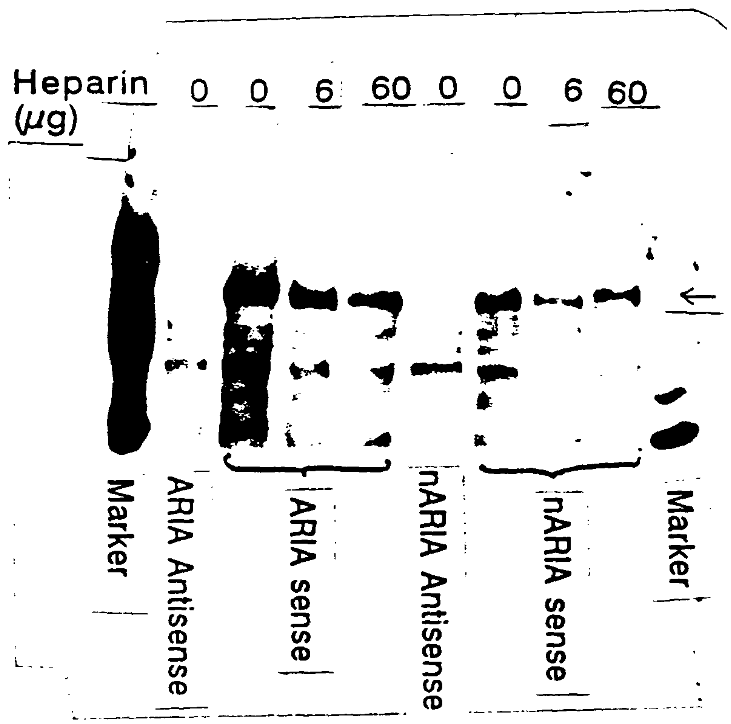

Heparin (µg)
0
0
6
60
0
0
6
60
Marker
ARIA Antisense
ARIA sense
nARIA Antisense
nARIA sense
Marker

FIG. 16

8% non-denaturing

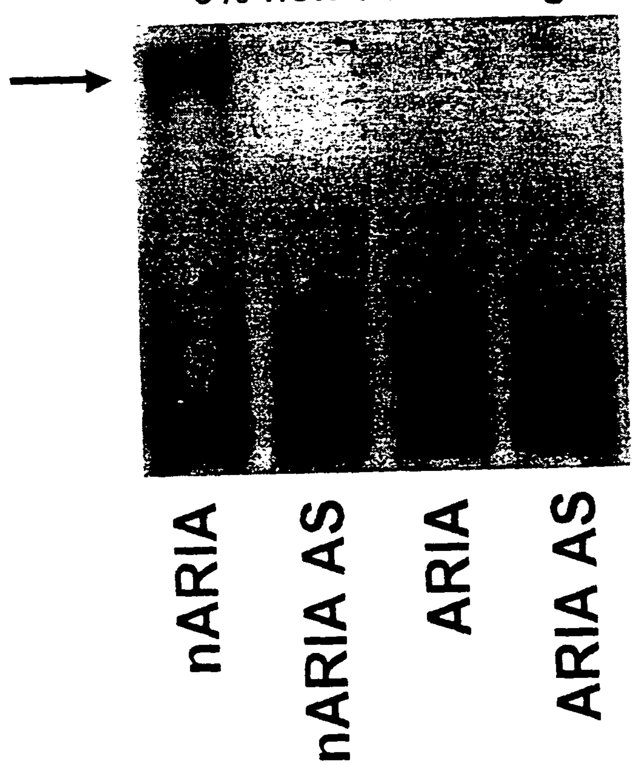


FIG. 17

FIG. 18

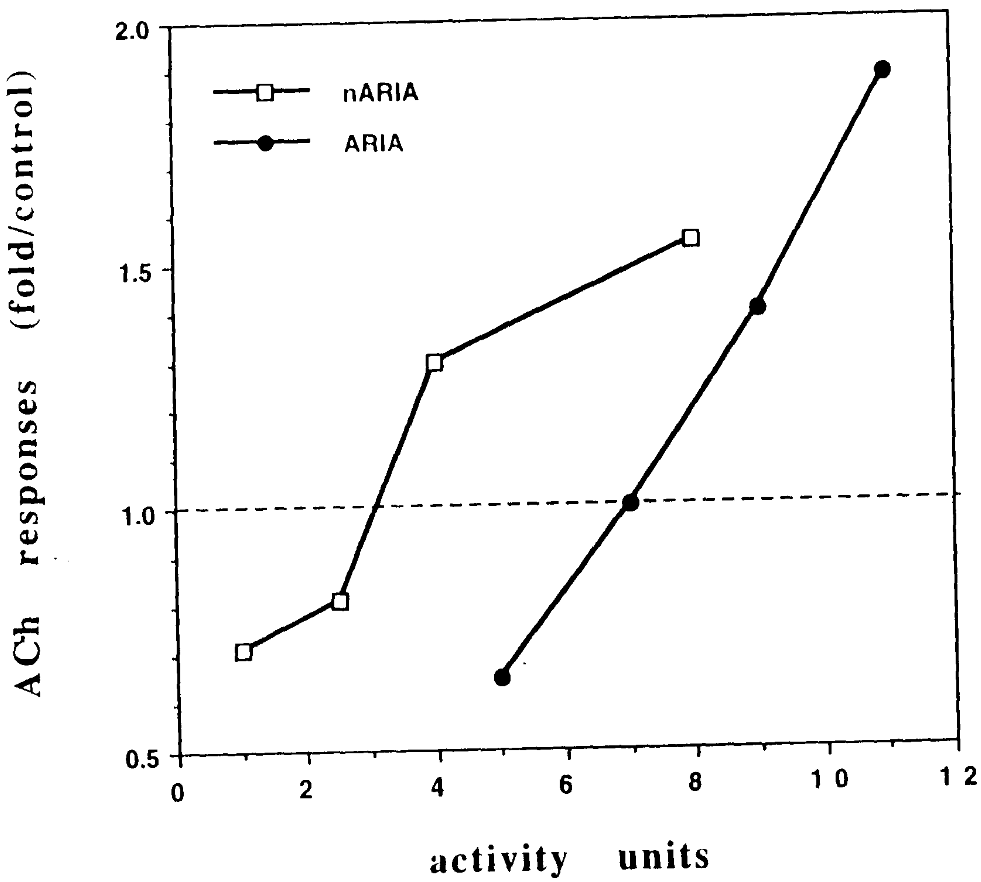
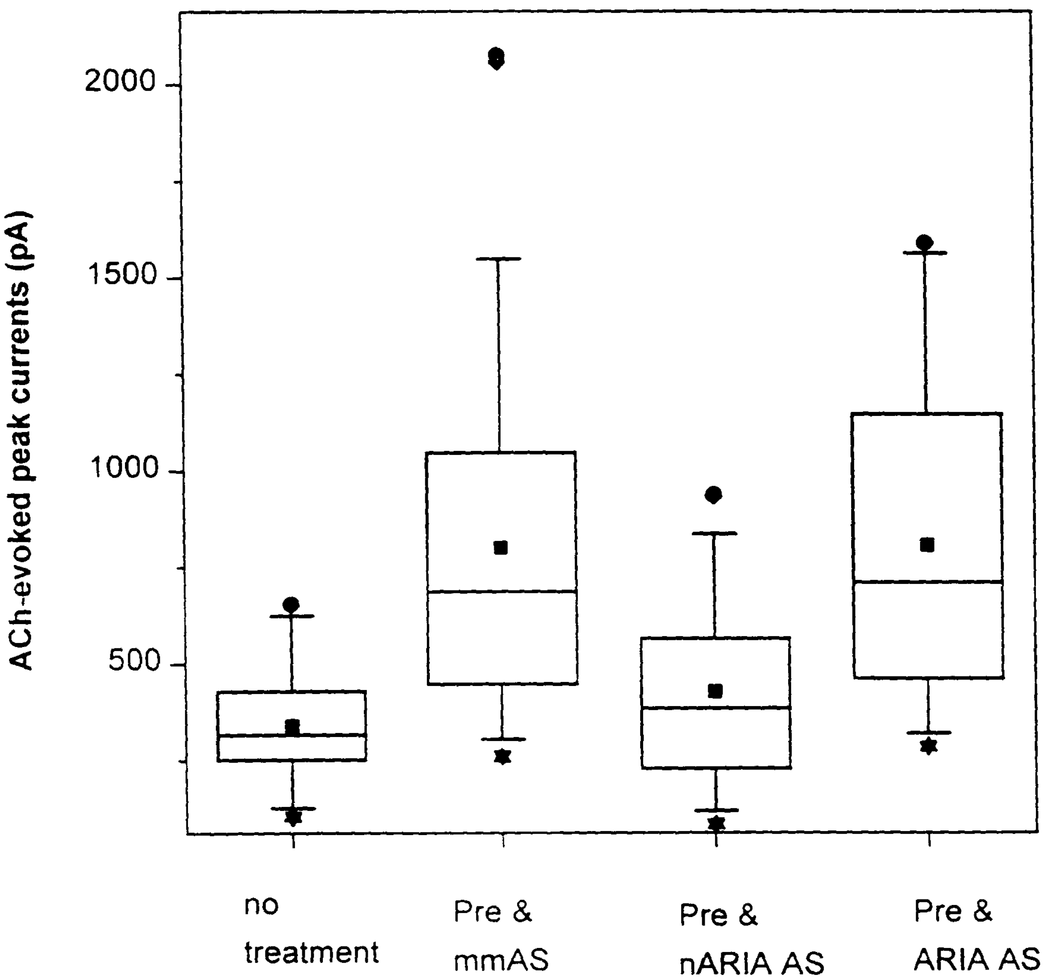

nARIA
ARIA
ACh responses (fold/control)
2.0
1.5
1.0
0.5
0
2
4
6
8
1 0
1 2
activity units

FIG. 19



no treatment=sympathetic neurons alone
'Pre'=treatment of sympathetic neurons with presynaptic input-conditioned media+various oligos
mmAS=mismatch antisense control
nARIA AS=nARIA specific antisense oligonucleotides
ARIA AS=ARIA specific antisense oligonucleotides

TABLE P1-1	α3	α5	α7	β4
SYMPs ALONE (set to ≡ 1) mRNA/SyMp (fg/100 fg std)	1.2 ± 0.2	0.4 ± .01	0.2 ± .05	0.4 ± 0.1
SyMps + INPUT	4.9	6.6	18	1.6
SyMps + TARGET	0.6	6.3	3.0	4.6
SYMPs+ INPUT+ TARGET	2.7	10	23	10
. *in vivo* DEVELOPMENT	2.8	11	21	12

Anterograde (Input) and Retrograde (Target) co regulation of nAChR expression utilize distinct (~ additive) mechanisms. nAChR mRNA were assayed from synaptically naive SyNs (E9 chick) *in vitro*. Conditions indicated & presented as fold change relative to E9 SyMps (≡ 1). n= (from top):49, 51, 17, 31, 6 experiments of each condition. Single cell qPCR following electrophysiology, data corrected for amplification efficiency & actin standard (& **Prog.: A2**) ^RNase protection assay of E8 vs. E21, corrected for neuron number and actin standard. + heart target data; # kidney target data (see **Aim 2 Progress**)

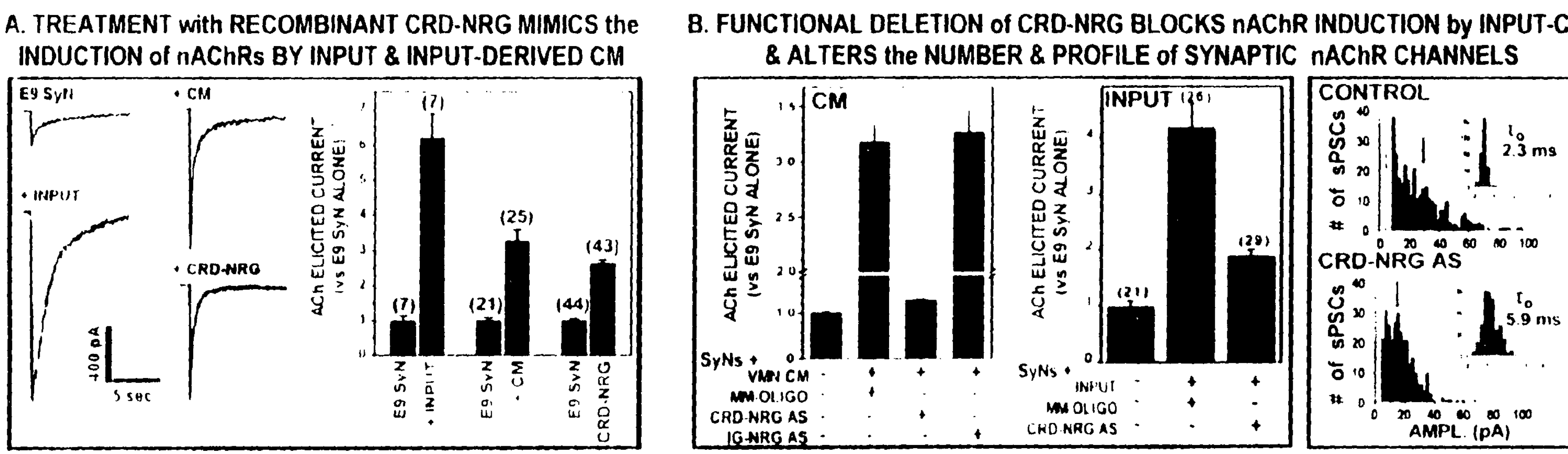
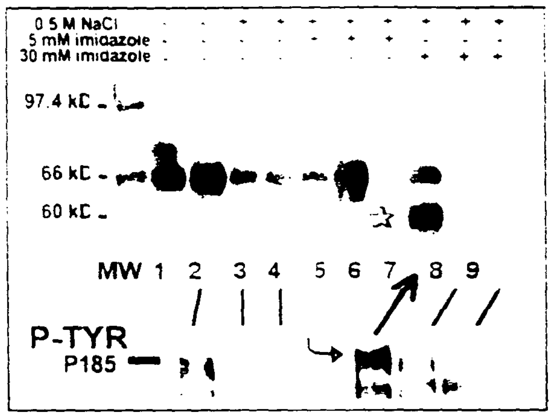
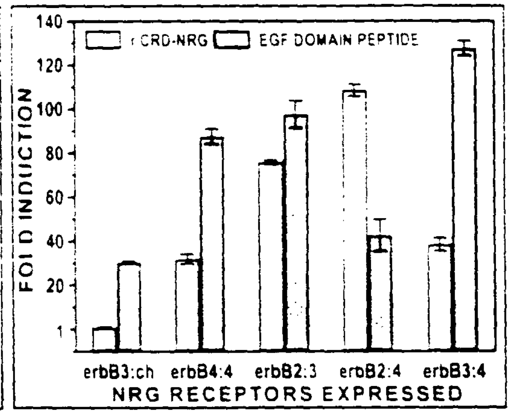


FIG. P1-1: Regulation of postsynaptic nAChRs by CRD-NRG in E9 chick sympathetic neurons.
Input-dependent induction of postsynaptic nAChRs is mimicked by CRD-NRG **(A)** and inhibited by CRD-NRG AS treatment **(B)**. CRD-NRG is required for nAChR induction by input-derived soluble factors (CM) since postsynaptic nAChR induction by VMN input is strongly inhibited by CRD-NRG AS (**B**: Middle). Analysis of synaptic currents (sPSCs) at CRD-NRG "deleted" synapses reveals that this NRG isoform is required for the expression of the mature array of high γ, brief τ_o nAChR channel subtypes, normally induced by VMN input (B: far right). SyMps innervated by CRD-NRG AS-treated VMN express long τ_o, immature nAChRs, akin to those detected prior to synaptogenesis. MM = mismatch (control) oligo



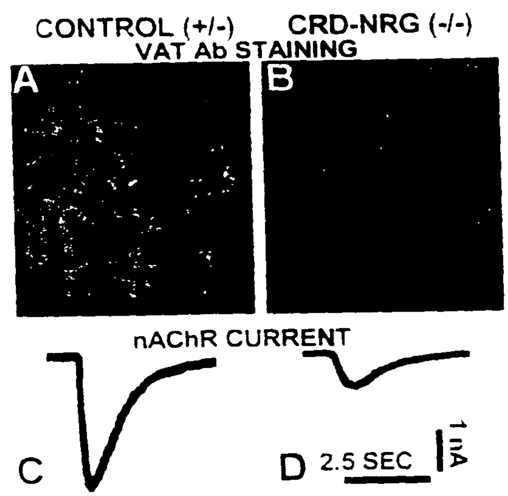


<u>FIG P1-2</u> **CRD-NRG biochemistry** Purification of recombinant CRD NRG (~75%; Lane 8, ~55 kD, by) and assay of enriched P185 P-tyrosine activity.

CRD-NRG activation of specific erbB combinations expressed in the 32D cell line. CRD is unique in its pattern of erb activation (vs. EGF peptide = Ig NRG).

29|54

Fig P1-3 VMN axons. within target SyMp. as seen with a VAT.Ab (+A) ,(B) By PO VAT+ axons and the ACh-elicited I_p are diminished in CRD NRG (-/-) vs control (+/-) mice.(C, D).



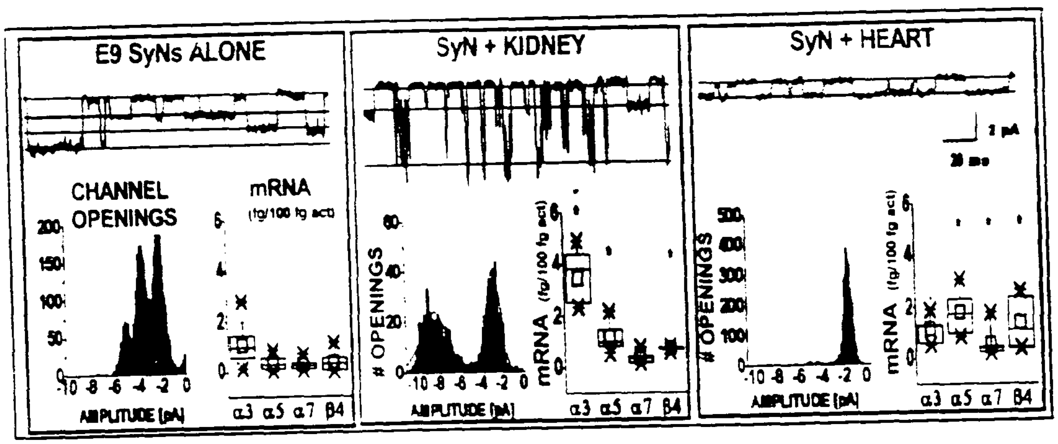

E9 SyNs ALONE
CHANNEL OPENINGS
mRNA
(fg/100 fg act)
AMPLITUDE (pA)
α3 α5 α7 β4
SyN + KIDNEY
OPENINGS
mRNA (fg/100 fg act)
AMPLITUDE (pA)
α3 α5 α7 β4
SyN + HEART
2 pA
20 ms
OPENINGS
mRNA (fg/100 fg act)
AMPLITUDE (pA)
α3 α5 α7 β4

FIG. P2-1

Fig. P2-2 Target specific regulation of synaptic nAChR channels in innervated SyMp

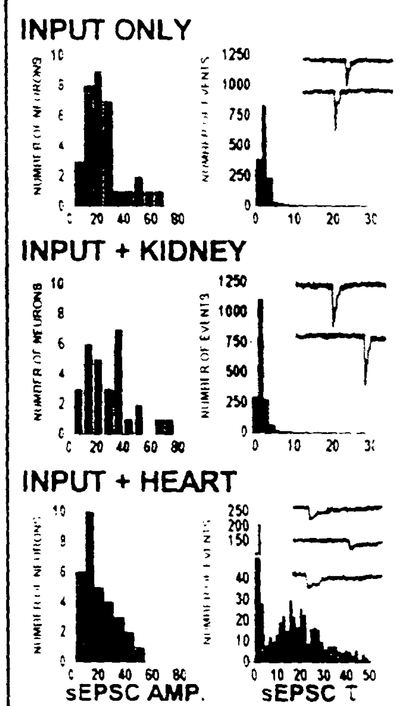


TABLE P2-1: Σ Regulation of nAChR s	$(\alpha3)_2(\beta x)_3$	$(\alpha3)_1(\alpha7)_1(\beta x)_3$	$(\alpha3)_3(\beta x)_2$	$(\alpha3)_1(\alpha5)_1(\alpha7)_1(\beta x)_2$	$(\alpha3)_2(\alpha5)_2(\beta x)$	$(\alpha3)_1(\alpha5)_2(\alpha7)_1(\beta x)$	α7
γ (pS)	13.5 ± 2	23 ± 3	28 ± 4	38 ± 6	50 ± 3	51 ± 3	66 ± 7
τo 1 (ms)	2.1 ± 5	1.1 ± 0.2	1.7 ± 0.1	1.7 ± 0.1	3.3 ± 0.5	3.3 ± 0.4	2.5 ± 0.8
τo 2 (ms)	7.6 ± 1 (65%)	7.0 ± 1 (60%)	13 ± 0.9	10.8 ± 0.4 (67%)	-	16.6 ± 3.1 (39 %)	
ABUNDANCE							
Early develop.	++++	-	++	-	+	-	-
Intermediate	+++	+	++	++++	+++	+++	+
Late develop.	-	++	-	++++	++++	++++	++
INDUCED by...							
Input	-		-	++	+++	++	+
Contact with kidney	-	-	-	+	++++	-	+++
Contact with heart	++++	++	+	-	-	-	-
PHARMACOLOGY	the number of + 's represents the relative apparent affinity for ACh (where > +'s indicates > apparent affinity)						
ACh (rel. K_{app})	++++	+++	++++	++	++	+	+
Cytisine[2]	+	-	+	-	+	-	ND
n-BgTx sensitivity	+	+	+	+	+	+	+
α-BgTx sensitivity	-	+	-	-	-	-	ND
MLA sensitivity[2]	-	-	-	+	-	+	+
DELETED by AS to :	α3	α3, α7	α3	α3, α5, α7	α3, α5	α3, α5, α7	α7 , ND

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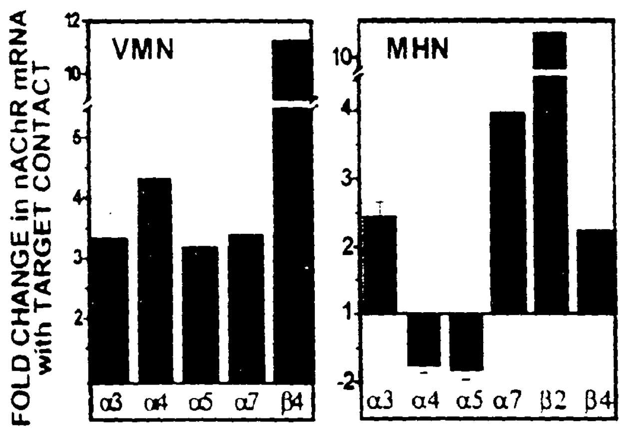
You have made it to **AIM 1 FIGS AND TABLES**

Table A1-1: **The profile of nAChR subunit gene expression in the visceral motor & medial habenula nuclei changes during *in vivo* synaptogensis.**

	$\alpha3$	$\alpha4$	$\alpha5$	$\alpha7$	$\beta2$	$\beta4$
Visc. Motor						
E18 vs. P0. mouse	↑	↑	-	↑	↑	**ND**
E 9 vs. E18. chick	**ND**	↑↑	↑	↑	↓	↑
Med. Habenula						
E16 vs. P0. mouse	↑	↑	↑	↑	↑↑	**ND**
E 11 vs. E17 chick	↓	–	↑	↑↑	↑↑	↑↑

qPCR assay of chick tissue extracts; mouse data from "side-by-side" in situ assays ([FN 1], Methods).
ND: not determined: - no change or low signal.

FIG A1-1: **The profile of nAChR subunit gene expression in presynaptic VMN and MHN neurons is strongly regulated by interaction with neuronal targets *in vitro*.**



nAChR subunit mRNA levels were assayed by qPCR of chick neurons *in vitro* ± synaptic partners.. The levels of subunit mRNA in "synaptically naive" neurons is set to 1.

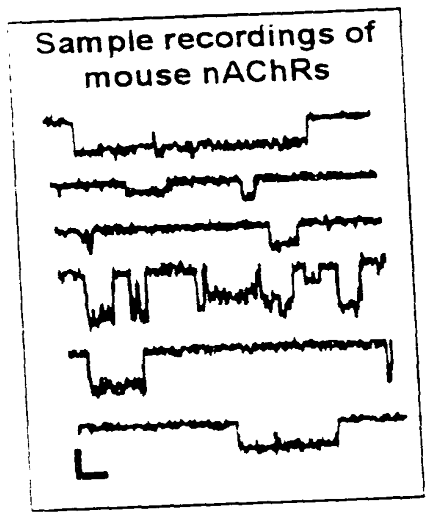

Sample recordings of
mouse nAChRs

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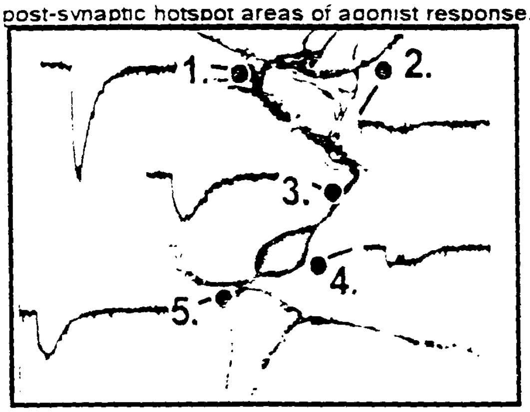
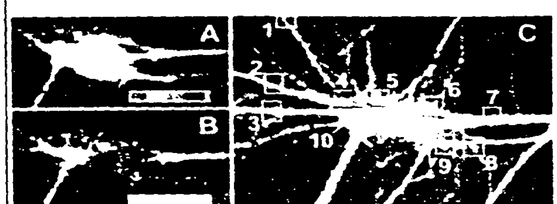
Fig A1-2 Mapping **post**synaptic "hotspots" by VC recording:
WCR and tests at 5 sites of n-n contact reveals post-synaptic hotspot areas of agonist response.



Mapping **pre**synaptic nAChR "hotspots" by fura2-imaging of nicotine-elicited [Ca2+] transients. Nicotine (1uM) was applied at 25 areas elicting increased [Ca] at 10 presynaptic hotspots (red filled circles & □ in C) . Blue /open circles areas tested. - after Mn quench.



Internal Mn perfusion of postsynaptic n. (A: & pseudo color purple) quenches somatic signal (B) and unveils presynaptic fibers (C: & yellow / green) calib: 0.2 fluor units x 20 secs)

TABLE A1-2	AChE Fibers	α3	α4	α5	α7	β2
IPN						
E16	+	-	-	+	-	++
E18/P0	+++	+/-	+	++	+/-	++
P7	+++	++	++	+++	++	+
AMYG						
E16	-	-	-	-	-	-
E18/P0	+ (gc)	+/-	++	+/-	+	+/++
P7	+++	ND	+++	+	+++	++

"Amygdala" refers to 2 major cholinoceptive subregions: the basolateral nucleus (BLA), and the Nucleus of the lateral olfactory tract nuclei (NLOT). (gc)= growth cone tipped AChE + fibers. ND= not determined.

Fig A1-3: *In vitro* innervation of IPN by MHN alters the profile of nAChR subunits expressed and increases the magnitude of nAChR-mediated currents

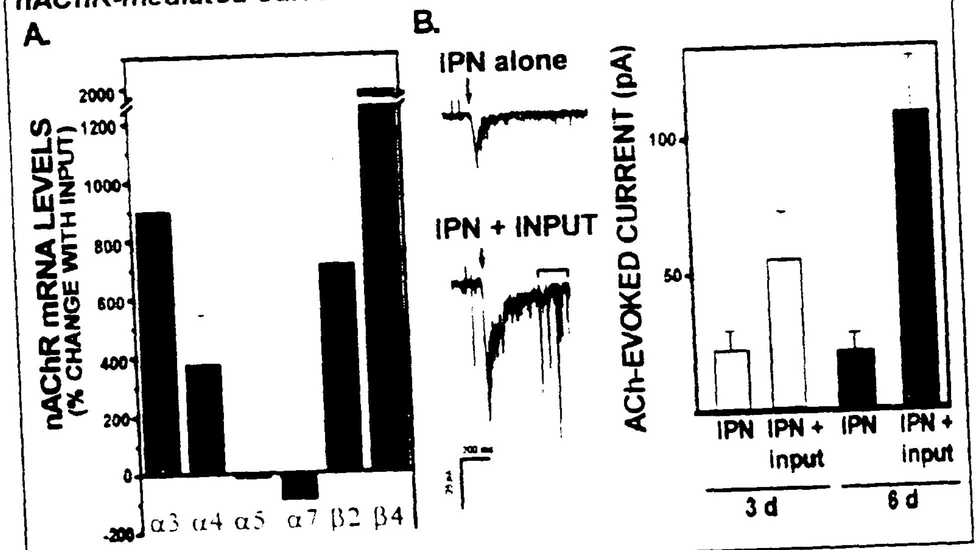


Fig A1-5: Nicontine induces robust synaptic facilitation: slice-patch recording from P0 mouse IPN (**B**). Neurons are dye filled for subsequent re-location (**A**)

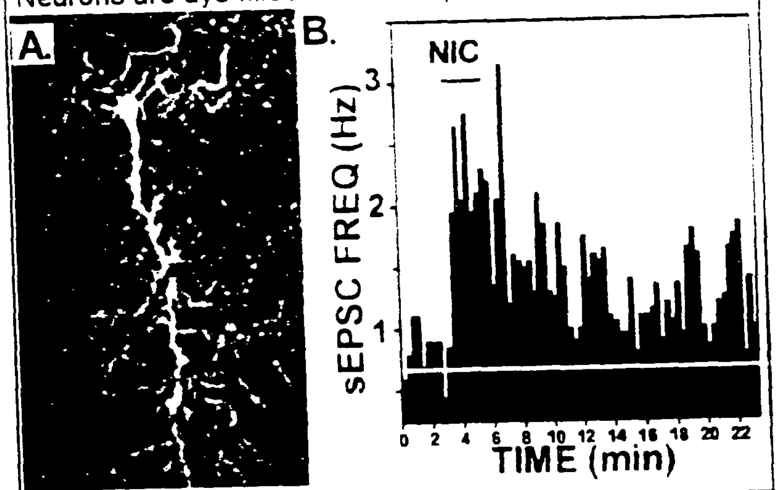


Fig A1-4: Cholinergic fibers exit mouse MHN micro-explants (VAT+: red)..Contacts with IPN MAP + dendrites are common (green).

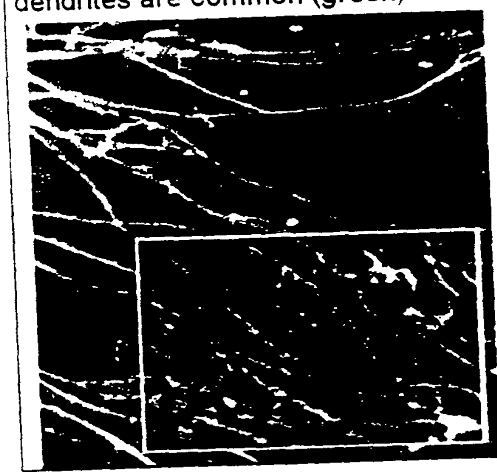
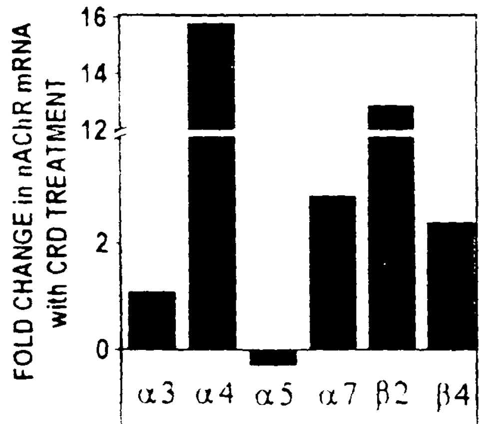


TABLE A2-1	α3	α4	α5	α7	β2	β4
VMNs *In vivo*						
(mouse)						
DEV. Δ's; E16 vs. P0	↑	↑	-	↑	↑	ND
CRD(-/-) vs. CONT	ND	↓↓	-	↓•	↓	ND
(chick) *in vitro*						
Δ with target	↑	↑↑	↑	↑	ND	↑↑
Δ with CRD NRG	↓?	↑	no	↑	ND	↑↑
MHN *In vivo*						
DEV. Δ's	↑	↑	↑	↑	↑↑	↑↑
CRD(-/-) vs. CONT.	noΔ	↓?	↓	↓	↓?	ND
in vitro						
Δ with target	↑	↓	↓	↑↑	↑↑↑	↑
Δ with CRD NRG	↑	↑	↓	↑↑↑	↑↑↑	↑↑
IPN *In vivo*						
DEV. Δ's	↑↑	↑	↑↑	↓	↑	↑↑
CRD(-/-) vs. CONT	#ND	↓	↓↓	no	no Δ	ND
in vitro						
Δ with input	↑	↑	↑?	↓	↑↑	↑↑
Δ with CRD NRG	↑	ND	↑?	↓↓	ND	–/?↑
AMYGD *In vivo*						
Δ DEV (P0 vs. P7)	ND	↑	↑?	↑↑↑	no Δ	ND
Δ in CRD(-/-), P0 m.	ND	↓	↓?	↓?	no Δ	ND
E16 mouse *in vitro*						
Δ with input	~↑	~↑	~↑	↑↑	no Δ	ND

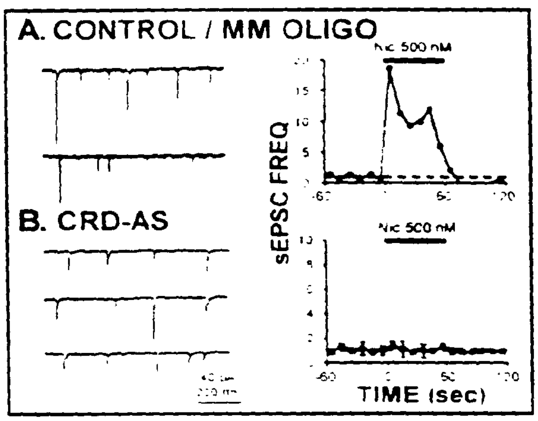
Mouse" *in vivo*" data refers to preliminary *in situ* analyses. All *in vitro* data refer ~~to~~ qPCR ~~assays~~ . ~~no~~ Δ : ~~no change in subunit~~ levels. **ND**= not determined ; ~ or ?: measurement uncertainty due to low "n" or low levels of expression. Also see Fig A2-2;

Fig A2-1:Recombinant CRD-NRG alters profile of nAChR subunit genes expressed in chick MHN neurons



nAChR subunit mRNA levels were assayed by qPCR of chick MHN neurons *in vitro*, treated (24 hrs) with rCRD-NRG or mock. The levels of subunit mRNA in mock treated neurons is set to 1.

Fig A2-2 CRD-NRG signaling may be required for expression &/or targeting of presynaptic nAChRs in CNS neurons



(A).Control: presynaptic nAChRs are present at VMN – SyMp synapses, as detected by increased sEPSC frequency (synaptic facilitation) with applied nicotine. (B)Treatment of VMNs with CRD-NRG AS-.(48 hrs) blocks nicotine-induced facilitation, although baseline synaptic activity (sEPSC freq.without nicotine) is unaffected. MM = mismatch (control) oligo

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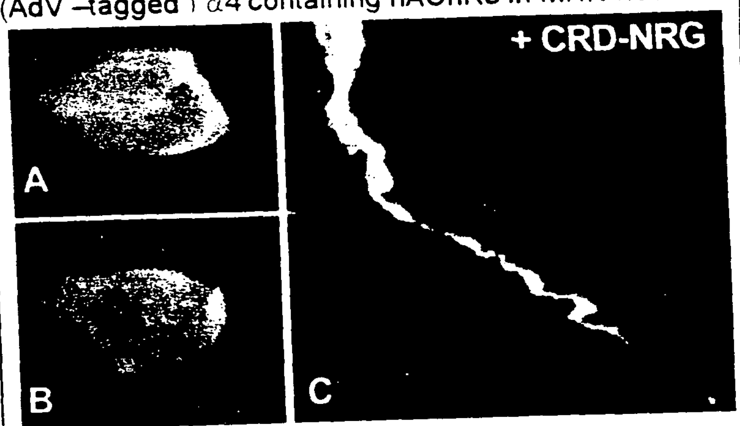
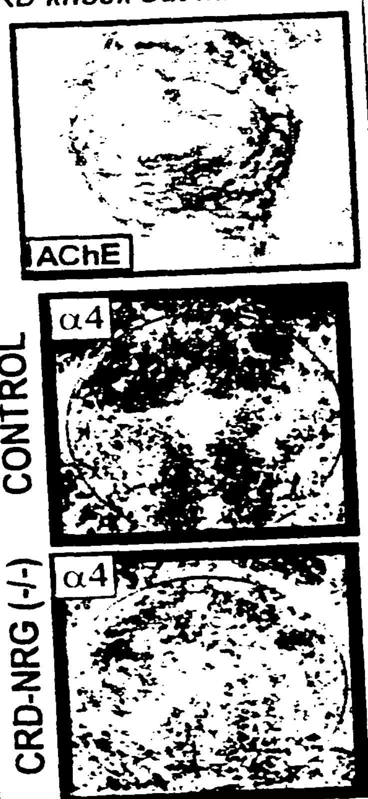
Fig A2-3 CRD-NRG ennances axonal targeting of (AdV –tagged) α4 containing nAChRs in MHN neurons



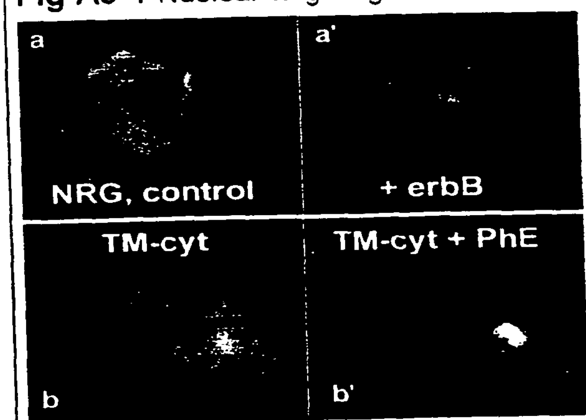
After Adenovirus mediated gene transfer of α4- FLAG in MHN, FLAG- nAChRs are seen only on the soma of control neurons,, whereas in CRD NRG treated (24 hrs). neurons. FLAG nAChRs are detected in MAP-minus neurites.

Fig A2-4: Altered pattern of $\alpha 4$ mRNA in the IPN of CRD knock out mice.

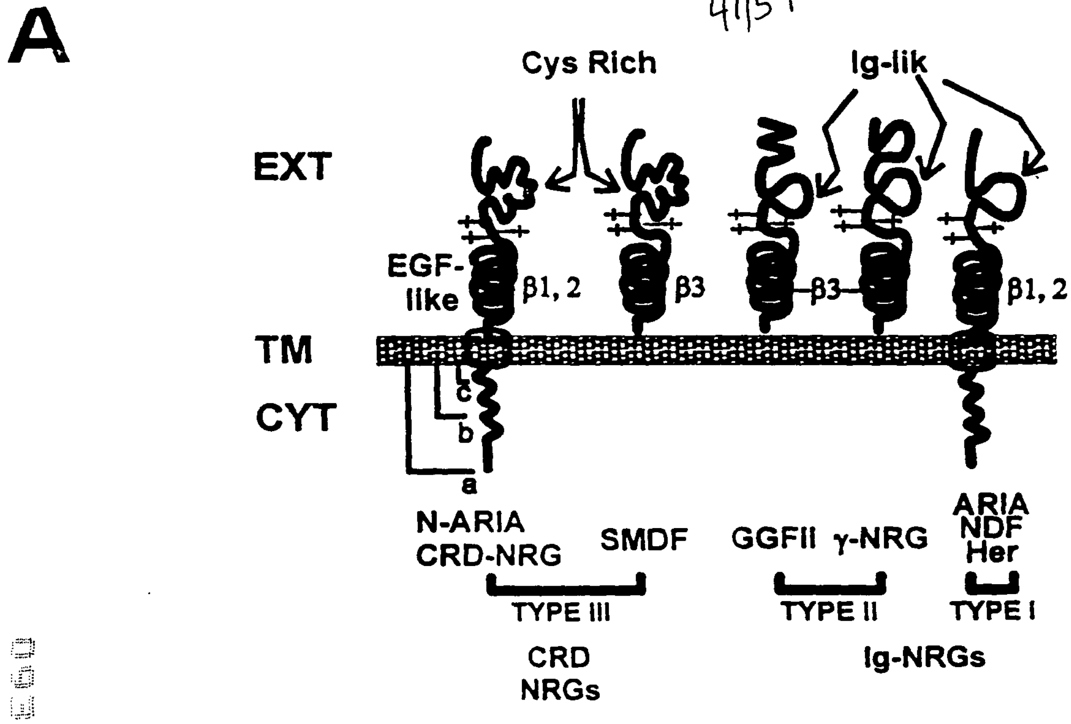


AChE stained fibers encircle the IPN. (top) Note IPN subregions differ in $\alpha 4$ probe *In situ*

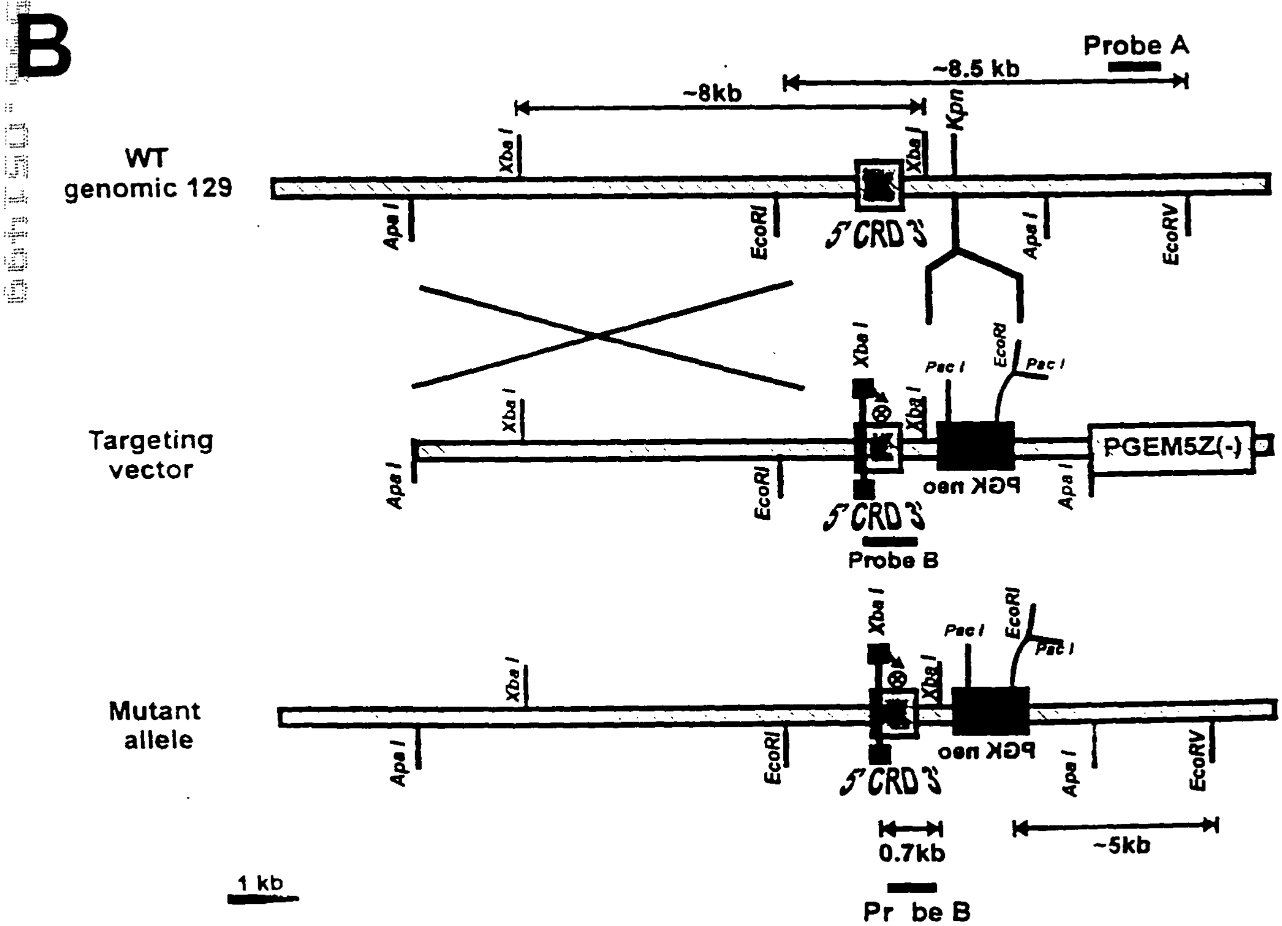
Fig A3-1 Nuclear targeting of NRG-CYT



Addition of erbB expressing cells (a, a') or activation of PKC (b,b') induces nuclear targeting of the cytoplasmic (CYT) domain from a full length NRG or a NRG transmembrane (TM) + CYT chimera



A
Cys Rich
Ig-lik
EXT
EGF-
like
β1, 2
β3
β3
β1, 2
TM
CYT
c
b
a
N-ARIA
CRD-NRG
SMDF
GGFII
γ-NRG
ARIA
NDF
Her
TYPE III
TYPE II
TYPE I
CRD
NRGs
Ig-NRGs



B
Probe A
~8.5 kb
~8kb
WT
genomic 129
Xba I
Xba I
Kpn
Apa I
EcoRI
5' CRD 3'
Apa I
EcoRV
Targeting
vector
Xba I
Xba I
Pac I
EcoRI
Pac I
PGEM5Z(-)
PGK neo
Apa I
EcoRI
Apa I
Probe B
Mutant
allele
Xba I
Xba I
Pac I
EcoRI
Pac I
PGK neo
Apa I
EcoRI
Apa I
EcoRV
0.7kb
~5kb
1 kb
Pr be B

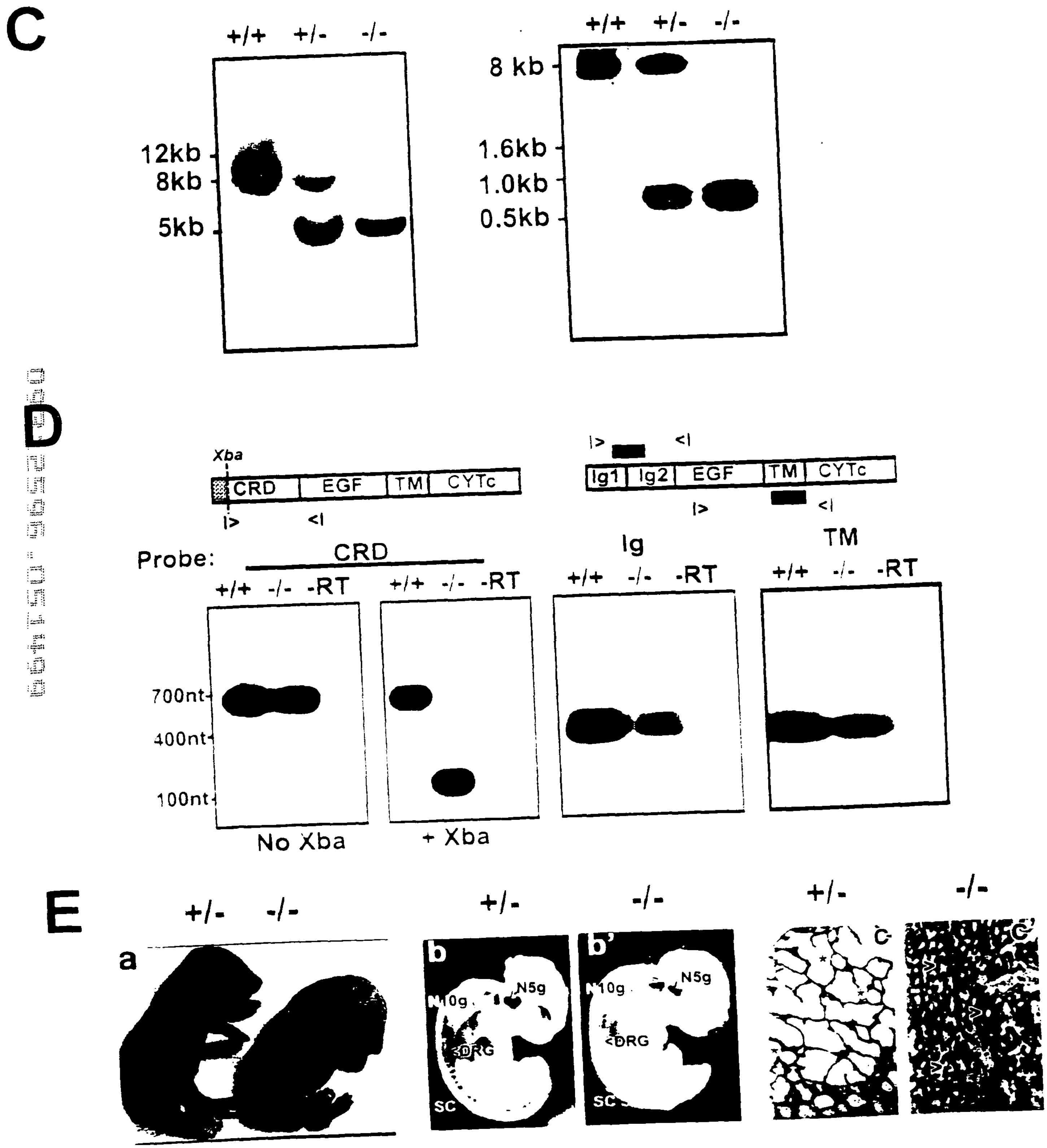

C
+/+ +/- -/-
12kb
8kb
5kb
+/+ +/- -/-
8 kb
1.6kb
1.0kb
0.5kb
D
Xba
CRD EGF TM CYTc
Ig1 Ig2 EGF TM CYTc
Probe:
CRD
Ig
TM
+/+ -/- -RT
700nt
400nt
100nt
No Xba
+ Xba
E
+/- -/-
a
b
b'
N10g
N5g
<DRG
SC
c
c'

FIGURE 2A Wolpowitz et al

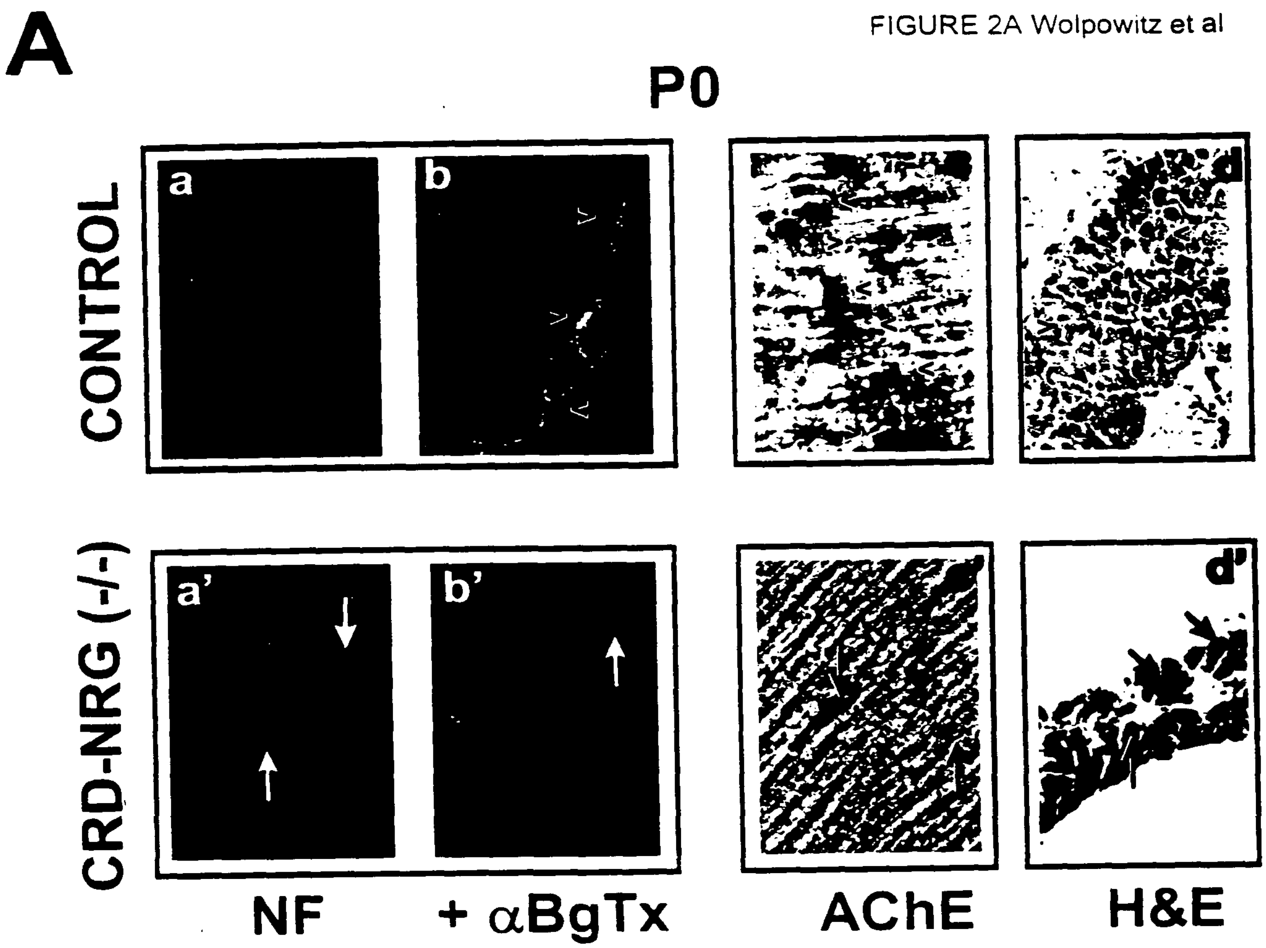


FIGURE 2B Wolpowitz et al

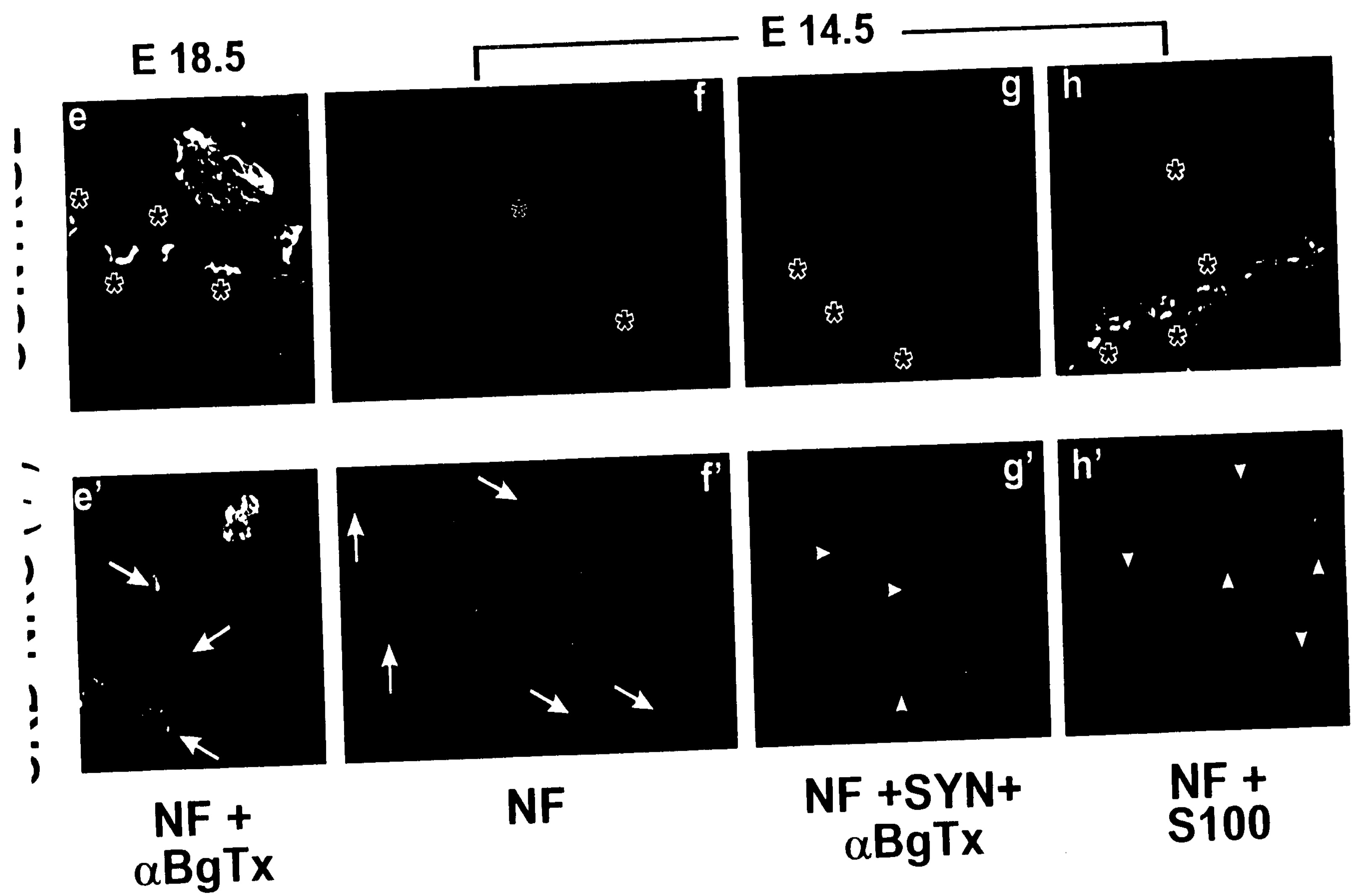
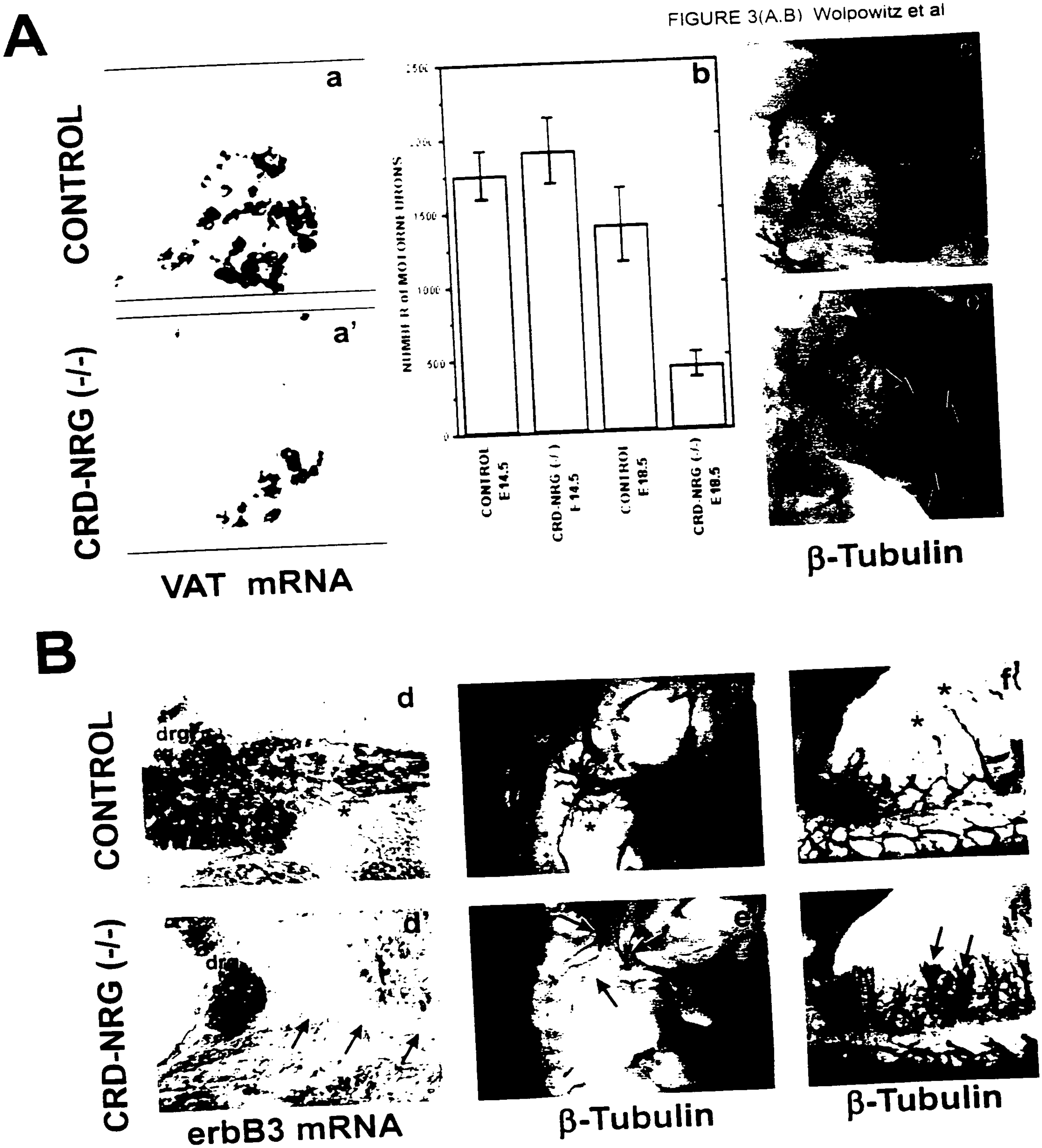


FIGURE 3(A.B) Wolpowitz et al



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C

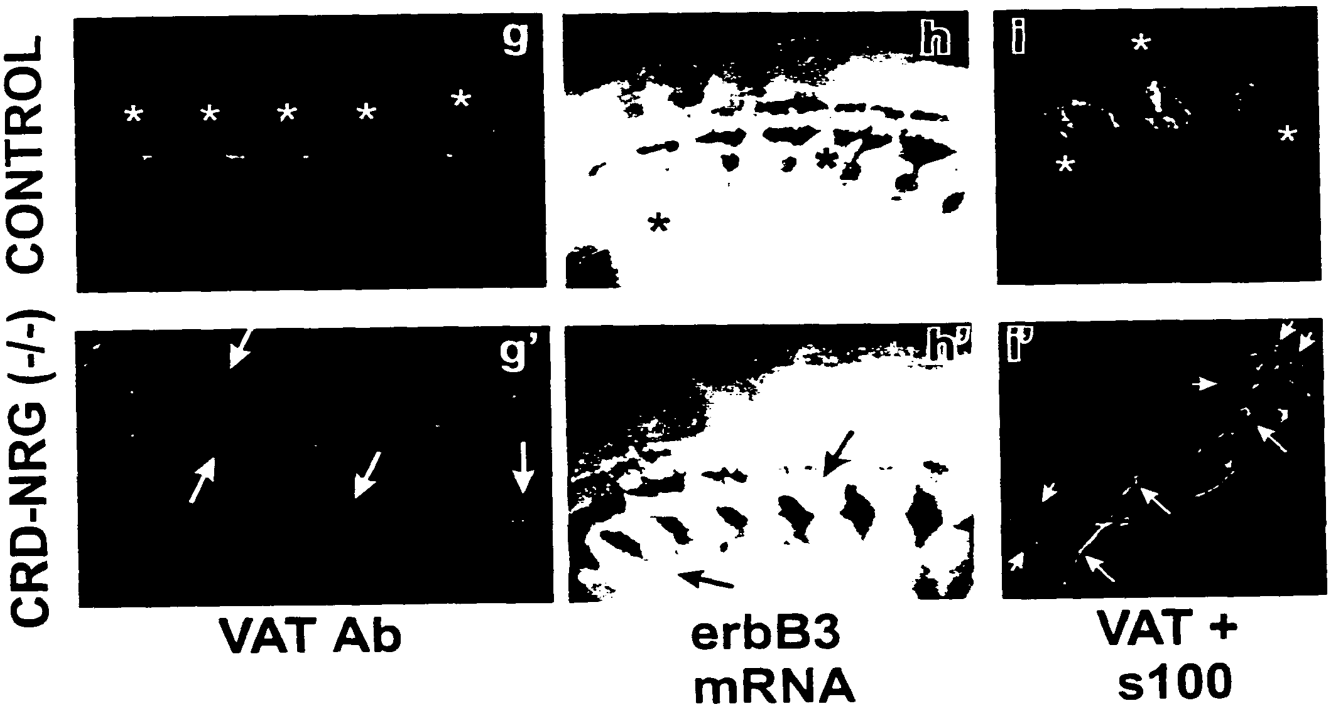

FIGURE 3(C) Wolpowitz et a

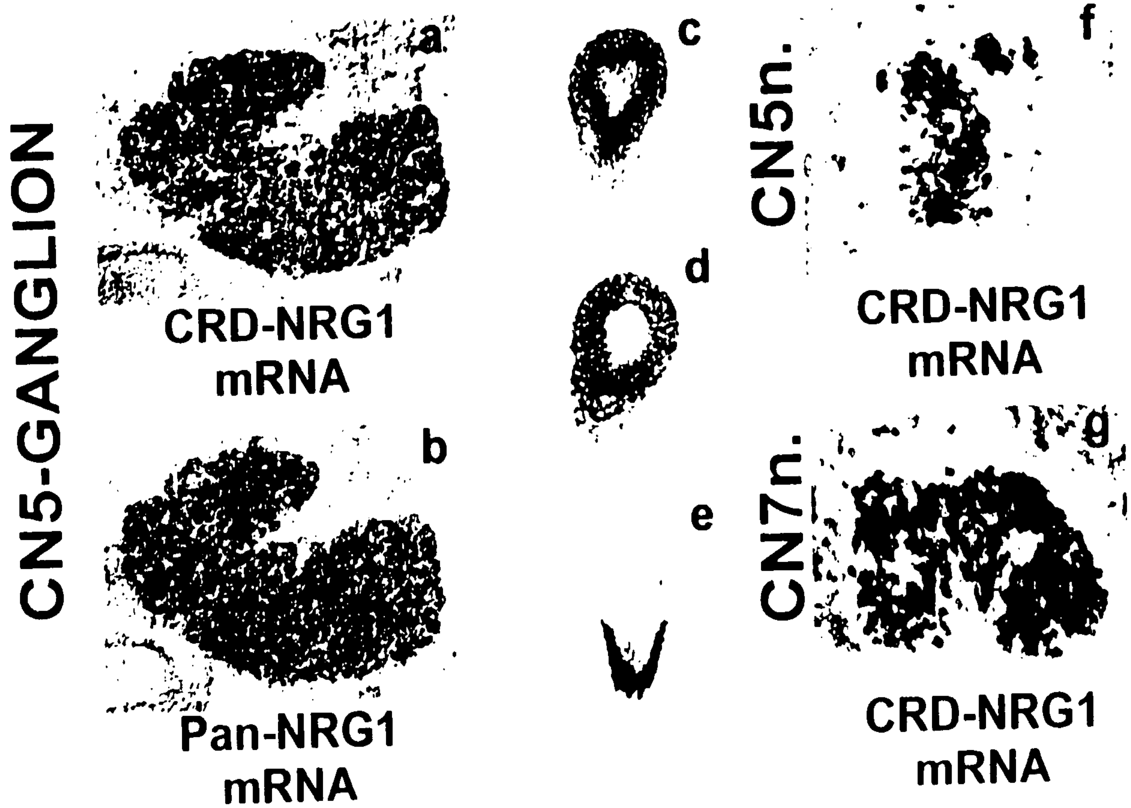
CN5-GANGLION
a
CRD-NRG1 mRNA
b
Pan-NRG1 mRNA
c
d
e
CN5n.
f
CRD-NRG1 mRNA
CN7n.
g
CRD-NRG1 mRNA

FIGURE 4B Wolpowitz et al